AN EVALUATION OF LOCAL ISOLATES OF *Hericium americanum* FOR USE IN MUSHROOM PRODUCTION

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ABSTRACT

The use of wild collected isolates of *Hericium americanum* (lion's mane) in the commercial production of mushrooms was investigated. Six isolates of *H. americanum* (He 1, He 2, He 3, He 4, He 5, and He 6) were collected from the Ithaca area in the fall of 2007. The *in vitro* vegetative growth of these isolates was compared to that of a commercial isolate of *H. erinaceus* (FFP3) on PDA at three temperatures (15^0C, 25^0C, 30^0C). We found that the fastest growth was displayed by some of the wild isolates at each temperature.

Three wild isolates of *H. americanum* (He 1 which had very fast *in vitro* growth, He 4, which had moderately fast *in vitro* growth and He 2 which had very slow *in vitro* growth) and the commercial isolate of *H. erinaceus* (FFP3) were selected to be grown indoors in supplemented sawdust (*Fagus grandifolia* or *Acer rubrum*) filled bags. A comparison of fresh and dry weight yields showed that the isolate with the fastest *in vitro* growth (He 1) did not have the highest yield of mushrooms as we had hypothesized.

For this production method holes were poked in the plastic bags to allow mushrooms to form on the outside of the bag, however, we observed fruiting inside the bag (FIB); a phenomenon in which malformed mushrooms form inside the bags. The weight of FIB was higher for the wild isolates compared to the commercial isolate. There was also an inverse relationship between the weight of FIB and yield for the wild isolates. When the weight of FIB and yield were combined (total yield) there was no difference among the wild and commercial isolates. This leads us to believe that if FIB could be reduced for the wild isolates the yields of those isolates would be comparable to those of the commercial isolate.

Post harvest characteristics were also investigated. Mushrooms from the four strains (He 1, He 4, He 2 and FFP3) had higher percent weight loss after the first three days of storage than is considered acceptable for the common button mushroom (*Agaricus bisporous*). If this weight loss is not acceptable for *Hericium* mushrooms newer storage methods should be investigated to reduce moisture loss in storage. A subjective quality rating was also used in postharvest evaluations of these isolates. In general, the quality ratings among the wild isolates were not different. Mushrooms produced by the wild isolates were never better than the commercial isolate.

Three wild isolates (He 3, He 4, and He 5) and the commercial isolate (FFP3) were grown outdoors on American beech (*Fagus grandifolia*) logs. The wild isolate, He 3, had the highest percentage of logs produce mushrooms (over 60%) compared to the other isolates (approx. 20%). The best prospect for wild *Hericium* isolates in commercial production appears to be in outdoor cultivation.

BIOGRAPHICAL SKETCH

The author was born and raised in Buffalo, NY. Her interest in plants was sparked by spending time at her grandparents' old farm house in West Seneca, NY. She was especially inspired by her grandmother who had the greenest thumb around despite no formal horticultural training. Jeanne attended SUNY College of Environmental Science and Forestry (ESF), majoring in environmental and forest biology and received a Bachelor of Science degree in 2003. She worked on the Asian Long-horned Beetle Eradication Program in NYC before beginning her studies for the Master of Science degree at Cornell University.

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CHAPTER ONE

INTRODUCTION

Lion's mane mushrooms (*Hericium spp*) are common residents of decaying trees and logs throughout the Northern United States and Canada, belonging to Hericiaceae, Basidiomycota (Kuo 2009). While members of the genus *Hericium* also occur in parts of Europe and Asia, there are three species of *Hericium* which occur in Eastern North America. They are *Hericum erinaceus, Hericium americanum*, and *Hericium coralloides.* All members of the genus produce white fruiting bodies (mushrooms) covered in downward cascading spines. In addition to being edible these mushrooms have been shown to have medicinal properties (Wasser & Weiss 1999, Jeng-.-Leun 2001, Moldavan 2007, Wasser 2002, Hobbs 1995, Abdulla 2008) making this fungus a prime candidate for the specialty mushroom market.

Specialty mushrooms can be defined as "economically significant mushrooms with the exception of *Agaricus bisporus*" (Royse 1999). The white button mushroom (*Agaricus bisporus*) is the most commonly grown mushroom in the United States, with more than 675 million pounds produced in 2007-2008 (Shepphard 2008). Specialty mushrooms such as oyster mushrooms (*Pleurotus ostreatus*), shiitake (*Lentinus edodes*), wine cap mushrooms (*Stropharia rugoso-annulata*), enoki (*Flammulina velutipes*) and lion's mane (*Hericium* spp.) have become increasingly popular with consumers. Specialty mushroom growers produced over 16 million pounds of mushrooms in 2007-2008. Within this category, shiitake production increased by 42% from the previous year (Shepphard 2008).

Besides being grown on a large scale in indoor production farms,

specialty mushrooms are also grown in agroforestry systems. Agroforestry is an intensive land management technique in which agricultural crops/animals are integrated with forested land (Garrett et al 1994). Forest farming is one specific type of agroforestry in which high value non-timber specialty crops are cultivated in the unique microclimate created by a forest canopy. Specialty mushrooms can be produced utilizing logs from trees that were cut as part of a forestry management technique called thinning. Forest farming is not only a way to produce forest products in an ecologically responsible way it also brings economic diversity and additional incomes to generally economically depressed rural areas.

With increased consumer interest in specialty mushrooms comes the need for new strains of these specialty mushrooms to further increase the yield of mushrooms per growing area for mushroom growers. While there are many available cultures of some specialty mushrooms for growers to choose from, such as shiitake and oyster mushrooms, other highly prized edible specialty mushrooms such as *Hericium* species do not have such a large library of cultures to choose from. While new strains can be "bred" from existing strains, studies have shown that wild strains that exist in nature have the potential to out-produce isolates currently being utilized in commercial growing operations (Uhart et al 2008).

Among *Hericium* species, only *Hericium erinaceus* is currently being grown at a significant commercial level in indoor facilities. We have no knowledge of any *Hericium* species being grown outdoors using forest farming techniques. Commercially, *Hericium* mushrooms are marketed under the common name "Pom Pom". The aim of this research is to investigate the potential for wild collected isolates of *Hericium americanum* in commercial

indoor production and in forest farming systems. This research will evaluate six wild strains of *Hericium americanum* collected in Ithaca, New York and the surrounding area for use in (i) indoor production of mushrooms as well as a preliminary evaluation for (ii) outdoor production in forest farming systems. The growth rate, mushroom yield and storability of mushrooms of the locally collected isolates of *Hericium americanum* will be compared to that of a commonly available commercial strain of *Hericium erinaceus*. Can one of these strains equal or outperform a currently popular commercial variety of *H. erinaceus*. If so, *Hericium americanum* may be incorporated into indoor commercial production operations as well as agroforestry mushroom production systems.

The specific objectives of this study are to:

1) Determine the differences in growth rate of the strains on PDA at different temperatures.

2) Compare yield and mushroom shelf-life (storability) between wild collected strains and a commercial strain on two different substrates when grown indoors.

3) Compare yield of wild collected isolates and a commercial strain when grown outdoors.

CHAPTER TWO

LITERATURE REVIEW

2.1 Lion's Mane Mushroom

The common name lion's mane applies to a number of species within the genus *Hericium*. *Hericium* species all produce large white fruiting bodies with downward cascading spines. In some species the spines arise from a branched base (*H. americanum* (Illustration 2.1) *and H. coralloides*) while others have spines directly attached to a more solid fleshy base (*H. erinaceus*) (Illustration 2.2). Within *Hericium* there are three species that are considered to be "exquisitely" edible (Stamets 1993) and they are relatively common throughout the Northeastern United States and Eastern Canada (Barron 1999). *Hericium erinaceus*, sometimes called the Pom Pom mushroom, is probably the most widely commercially available species, while *Hericium americanum* and *Hericium coralloides* are less frequently available for commercial sale.

Besides being choice edibles, *Hericium* mushrooms contain compounds that may have beneficial medicinal properties. Extracts form *Hericium* mushrooms have been shown to have antitumor, immunomodulating (Wasser & Weis 1999), antioxidant (Jeng-Leun 2001) and nerve cell-stimulating effects (Moldavan 2007). Extracts have also been used to treat ailments such as chronic bronchitis (Wasser 2002), hepatitis B (Hobbs 1995) and a range of gastrointestinal problems (Abdulla 2008). Due to their delicious flavor and medicinal properties, *Hericium* mushrooms have become a high value mushroom variety which typically sells for considerably more than other specialty forest mushrooms, i.e. shiitake and oyster.

Illustration 2.1 Mushroom from *Hericium americanum*

Illustration 2.2 Mushroom from *Hericium erinaceus*

Unfortunately for those who wish to grow *Hericium*, there are few strains available when compared to the wide range of strains of other varieties of mushrooms, such as oyster and shiitake. One popular mushroom spawn supplier carries 12 strains of shiitake and 6 strains of oyster mushrooms, while only offering one variety of one species of *Hericium* (www.fieldforest.net). A survey of the culture collection maintained by Penn State's Department of Plant Pathology shows availability of two strains of *Hericium coralloides*, two strains of *Hericium erinaceus*, no available strains of *Hericium americanum* and an astounding 117 strains of Shiitake (*Lentinula edodus*) (http://mushroomspawn.cas.psu.edu/CultureList.shtml). Strains of other *Hercium* species, like *H. americanum* and *H. coralloides*, are virtually impossible for growers to purchase.

2.2 Markets for Specialty Mushrooms

Despite the fact that mushrooms are considered impulse buys and are highly perishable compared to other kinds of fresh produce, their increase in popularity among consumers has brought them from the back of the produce department into the forefront. The most common mushrooms found in the food markets and restaurants of North America are the white button mushroom (*Agaricus bisporus*). These mushrooms are familiar to consumers, however, specialty mushrooms, such as oyster mushrooms (*Pleurotus ostreatus*), shiitake (*Lentinus edodes*), wine cap mushrooms (*Stropharia rugoso-annulata*), enoki (*Flammulina velutipes*) and lion's mane (*Hericium* spp.) are less commonly found in retail stores and therefore are less familiar to consumers.

As of 1991 Phillips Mushroom Farms, one of the largest growers of

specialty mushrooms in the United States, reported that only 5% of current sales are to retail markets and 95% of sales are to bulk wholesale markets. Retail markets are seen as the area with the greatest potential for growth (Donovan 1991). As consumers become more familiar with ethnic foods the demand for specialty and exotic mushrooms has increased as consumers have tried to recreate favorite restaurant dishes at home. Specialty mushroom sales are generally more in urban and cosmopolitan areas where the consumer base has more disposable income to buy these higher priced items in addition to being more familiar with ethnic dishes that use these mushrooms as ingredients. Retailers that have a customer base with a strong Asian influence also can expect high sales of specialty mushrooms because of the culture's appreciation for and use of exotic mushrooms in cooking (Gunn 1992). Specialty mushrooms can be sold in bulk but are typically sold in small packages weighing approximately 100g (Gunn 1992). Packages may also contain recipes and or other serving suggestions designed to coax more mycophobic consumers into trying new mushroom varieties (Donovan 1991). The price is considerably greater than that of more common mushrooms like button or portabella (*Agaricus bisporous*).

The biggest downside of growing specialty mushrooms is that there is not really a secondary market. Secondary markets are used in produce production when there is overproduction of the product such that the primary market cannot be the sole outlet. Other mushrooms and produce can be canned or frozen while the only real option for specialty mushroom is to sell them dried. However, this is not a very attractive option for growers because the monetary return on dried mushrooms can be fairly low. Since mushrooms can be 50-90% water, the dramatic decrease in weight and value that occurs

during the drying process in addition to the additional processing of drying has caused some growers to prefer to just dispose of the extra product as a way to deal with the problem of overproduction (Donovan 1992). This lack of a viable secondary market means that new varieties of specialty mushrooms need to have good fresh storage qualities to ensure they can be sold in the primary market.

Many different varieties of specialty mushrooms are currently being used as part of forest farming operations. Oyster mushrooms (*Pleurotus ostreatus*), shiitake (*Lentinus edodes*), wine cap mushrooms (*Stropharia rugoso-annulata*), and lion's mane (*Hericium* spp.) are merely a few that have been cultivated thus far. Lion's mane mushrooms (*Hericium* spp.) are the focus of this project.

2.3 Mushrooms in Agroforestry

Agroforestry is an intensive land management technique in which agricultural crops/animals are integrated with forested land (Garrett et al 1994). Forest farming is one specific type of agroforestry in which high value non-timber specialty crops are cultivated in the unique microclimate created by a forest canopy. The specialty crops provide the land manager with a short term income while waiting for the timber value of the site to be realized. Forest farming as an agroforestry technique is not only a valuable economic venture; it is a way for land owners to produce agricultural products from land with minimal environmental impact (Rietveld 1996). According to Bill Rietveld, program manager of the US National Agroforestry Center, agroforesty practices can aid in reducing erosion, protecting terrestrial and aquatic wildlife, improving water quality, and enhancing local biodiversity and landscape

diversity (Rietveld 1996).

Many specialty products can be produced in forest farming systems. Examples of such products are gourmet and medicinal mushrooms. The log-based production of mushrooms can utilize the waste wood generated through forest stand improvement operations, i.e. stand thinning. This wood is not timber quality and could be sold for firewood. However, a significant increase in profit might be seen if the logs are used as a substrate to produce high value specialty products like gourmet or medicinal mushrooms. The use of mushroom production as a forest farming system can increase income, increase product diversity of rural areas and allow small scale farmers to take an active interest in the management of their forested land. There are currently approximately 500,000 nonindustrial forest owners in New York State, any number of which could benefit from mushroom production (www.dnr.cornell.edu Apr 2008).

2.3.1 Use of Local Hericium Ecotypes in Agroforestry systems

An ecotype consists of a population of individuals within a species that have become adapted to a set of local environmental conditions through the process of natural selection. Ecotypes show that there are genetic differences between individuals for geographically different populations. Local varieties of *Hericium* might be better adapted to growing in this climate compared to the commercial *H. erinaceus* strain which is probably of Asian origin (Joe Krawczyk, personal communication). Thus local strains may produce higher yields of mushrooms compared to commercial strains in outdoor growing conditions.

Early experiments with log-based production of *H. erinaceus* here at

Cornell University (unpublished results) have shown the commercial *Hericium erinaceus* strain to be slow growing, and producing few fruiting bodies within the first two seasons. This may be because the *Hericium* strain that is commercially available to growers is either not well suited to the local climate or because it is a strain that is not well suited to current log-cultivation techniques.

Another reason for using local strains in agroforestry production of mushrooms is that growers may not wish to release Asian strains of *Hericium* into NY forests. The dangers of non-native invasive species of plants and animals are well known to members of the conservation world. Could the introduction of foreign strains of mushrooms, such as *Hericium* sp, have a negative impact on the local populations of *Hericium* sp or the forest community as a whole? While there has never been a *published* report of naturalization of probably the most commonly grown forest mushroom, shiitake (*Lentinus edodes* originated in Japan), there have been anecdotal accounts of this occurring (Joe Krawczyk, personal communication).

While *Hericium* spp. are generally described as saprophytes, results from a study by Filip et al. (1984) suggest that at least one *Hericium* spp. may be slightly pathogenic. The researchers were focusing on decay in Pacific Silver Fir (*Abies amabilis*) as it relates to economic loss on timber stands when they discovered the greatest proportion of decay (34.4%) was caused by *Hericium abietis*. Much of this decay was not associated with wounds which led the researchers to consider *H. abietis* as a possible pathogen or endophyte. If one member of the genus has been suspected of having pathogenic tendency without proper study it is not advisable to introduce nonnative fungal strains from Asia to North America.

2.4 Indoor Mushroom Production

The method for indoor production of mushrooms varies depending on the species of mushroom being grown. White button mushrooms, crimini and portabello (all *Agaricus bisporous)* mushrooms are grown on open beds of composted straw and manure and later cased with a sterile topping to promote fruitng. Enoki mushrooms (*Flammulina velutipes*) and king oyster mushrooms (*Pleurotus eryngii)* are commonly grown in plastic bottles filled with supplemented sawdust. Oyster mushrooms (*Pleurotus ostreatus*) are grown in long plastic bags filled with straw. *Hericium* mushrooms, the focus of this study, are grown in polyethylene bags filled with sawdust supplemented with a nitrogen-rich additive such as rice bran, wheat bran, or other agricultural waste products. The bags typically have small micro porous breathing patches that allow for gas exchange. Generally the bags are filled with the supplemented sawdust mixture and sterilized to kill bacteria and molds that would compete with the desired fungus. Once the bags of substrate are sterilized, the fungal spawn of the desired mushrooms is added to the bag. The bags are then sealed, the contents are mixed and they are set in the growing room to incubate. Specific humidity, temperatures and CO_2 levels are maintained in the growing room to optimize fungal growth. For *Hericium* a temperature of 21-25^{0}C, relative humidity of 95%, and CO_2 levels between 5000 - 40,000ppm is recommended to encourage mycelial growth. When the mycelium of the fungus has satisfactorily colonized the substrate, a few holes are poked in the bag with the expectation that the mushrooms will emerge at those sites. To encourage primordia, (the precursors of mushrooms) to form, the environmental conditions are altered to a cooler temperature of 16-18^{0}C and full fresh air (to decrease the CO_2 levels to 500-700ppm), as well as addition

of 500-1000 lux of light on a 12 hour cycle. Once primordia have formed the temperature can be raised to a range of 18-24^{0}C. (Stamets 1993) This is generally accomplished by having two separate growing rooms, one set up to encourage mycelial growth and mushroom growth and another to encourage primordia formation.

2.4.1 Use of Wild Local Strains of Hericium in Indoor Production

In a study by Xiao and Chapman (1997) fruiting bodies of *Hericium abietis* was collected from the wild and established in culture on potato dextrose agar (PDA). *H. abietis* is found in the Pacific Northwest of North America on conifer logs. In this study the newly established culture of *H abietis* and a commercial strain of *H. erinaceus* were grown out in bags of conifer sawdust. Each bag inoculated with the *H. abietis* culture successfully produced mushrooms, but the yield of mushrooms (by weight) was less than that of the yield produced by the bags inoculated with *H. erinaceus*. This shows that cultures from the wild can successfully be cultivated indoors on sawdust. While the yield of mushrooms for the wild strain of *H. abietis* was less than that of *H. erinaceus* this study examined only one wild isolate of *H. abietis*.

A similar study by Ko (2004) comparing mushroom yield of various species of *Hericium* including *H. americanum* (1 isolate) demonstrated that indoor cultivation of *H. americanum* and *H coralloides* could be accomplished using supplemented sawdust as a substrate. They suggest that “further experiments with diverse strains of these two species need to be preformed to improve their productivity and biological efficiency”.

Perhaps with a larger number of wild strains and improved cultivation

methods a strain of *H. abietis* or *H. americanum* could be shown to surpass the production of commercial strains of *H erinaceus* as has been seen in other species of mushroom. For example, a study by Uhart et al (2008) explored the use of wild strains of *Agrocybe cylindracea* for production. They found that the highest biological efficiency (fresh weight of harvested mushrooms / dry weight of substrate) was found in three of the eight wild strains and two of the four commercial strains used in the study. They also found that the positive effect of substrate supplementation was stronger in the wild strains. It caused one strain to display 179% bioefficiency, which is the highest known for the species (Uhaet et al 2008). These results clearly show the importance of searching for and evaluating naturally occurring wild strains of edible mushrooms.

2.5 Testing New Strains for Production

2.5.1 Growth rate

According to Stamets (2000), there are no fewer than 28 characteristics one should consider when selecting a new mushroom strain for production. One of the most important characteristics is rate of mycelial growth. This growth rate corresponds to the rate of substrate colonization which has direct economic importance when evaluating strains for commercial production. The length of time it takes the fungus to colonize the substrate has an impact on the productivity (bioefficiency/day). Rapid colonization reduces length of incubation time prior to fruiting, which results in more efficient use of production space. In addition, the substrate that remains uncolonized by mycelium remains vulnerable to mold and bacterial contaminants that could ultimately reduce yield (Zervakis 2001).

In a study by Ko. et al (2005) the growth rate of nine *Hericium* isolates

of seven species (*H. abietis, H. alpestre, H. americanum, H. coralloidies, H. erinaceus, H. lacinaceum, and H. erinaceum*) were examined. They first explored the effect of temperature on mycelial growth on PDA (potato dextrose agar) among the nine isolates. They found that the maximum growth rate of the isolates varied between isolates of different species as well as isolates of the same species. *Hericium americanum* was found to have the fastest growth rate among the species with a maximal growth rate 30^0C. *H. erinaceus* reportedly showed the fastest growth rate at 25^0C. A study by Ginns (1985) similarly showed a wide variation in the growth rate of several cultures of *Hericium* species at varying temperatures. In part the study compared the growth rate of *H. abietis* (16 isolates), *H. americanum* (11 isolate), *H. corallodies* (3 isolates), and *H. erinaceus* (12 isolates) on malt agar. The results showed considerable variation in growth rates between as well as among the isolates. *H. coralloides* and *H. americanum* had the fastest maximum growth rate while *H. erinaceus* and *H. abietis* had the slowest maximum growth rate. Ginns (1985) found 25^0C to be the optimal temperature for most *H. americanum* isolates and 30^0C to be optimal for most *H. erinaceus* isolates, they also concluded that the cultures "showed a considerable variation among the cultures." For example, after growing cultures of *H. americanum* for 18 days at 25^0C the colonies ranged in temperature from 17mm to 83mm.

Temperature was shown to have an effect on growth rate. Contrary to what was reported in Ko et al (2005) Ginns (1985) found *H. americanum* had the fastest growth at 25^0C as opposed to 30^0C, while *H. erinaceus* grew fastest at 30^0C, rather than 25^0C. When Imtiaj (2008) compared the growth rate of 4 strains of *H. erinaceus* the researchers found 25^0C and pH 6 to be

the optimum conditions for growth. These contradictions show that more work needs to be done to determine the optimum temperature for the growth of *Hericium* species. It seems that optimum temperature varies with individual as well as species. The effect of temperature on fungal growth was also explored in a study comparing the growth rates of *Beauveria bassiana* isolates, an entomopathogenic hypomycete. In the study, Fargues et al. found that "relative growth rates were not very useful in distinguishing differences among many isolates at the near optimal temperatures." They further found that differences in relative growth rate could more easily be determined as the temperature deviated above or below the optimal temperature (Fargues 1997). These results along with the results of Ginn (1985) and Ko (2004) suggest when comparing growth rates of new fungal isolates intended for use in mushroom production it may be advisable to compare the isolates at more than one temperature.

The study by Ko et al (2004) also looked at the effect of supplementation to sawdust substrate on mycelial growth rate of the same nine *Hericium* isolates. Researchers supplemented oak sawdust with rice bran, barely bran, soybean powder, eggshells, Chinese cabbage or wheat bran. It was shown that the relative growth rate of isolates within the same species of *Hericium* differ depending on the supplement given. Isolate 1101 had the fastest growth rate with all supplements except when supplemented with Chinese cabbage, in which case isolate 1008 had the fastest mycelial growth rate. In a study by Lee et al (2004) of six strains of the edible fungus, *Sparassis crispa*, researchers found one strain had maximal growth rate on a type of sawdust different from that of the other strains. The fastest mycelial growth was seen on larch sawdust except for one strain that showed the

fastest growth on oak.

These previous studies suggest that if newly collected fungal strains are to be tested for use in mushroom production, growth rates of the strains should be compared on PDA (potato dextrose agar) at varying temperatures. Growth rates on PDA (potato dextrose agar) is a conventional and convenient was to determine growth rates of fungal cultures. Another option would be to test the growth rates on the sawdust substrate, however if we find the growth on PDA correlated with yield it could become a quick, easy and standard way to screen new cultures.

2.5.2 Indoor Mushroom Production on Sawdust

Since the main goal of selecting mushroom strains is to produce fruiting bodies another characteristic to consider in new mushroom strains is fruiting ability. This is measured in terms of mushroom yield. For these experiments fresh weight yield is defined as the total fresh weight of all mushrooms produced per bag. Dry weight yield is the total oven dry weight of mushrooms produced per bag.

It is uncertain if strain performance of indoor cultivation on sawdust is indicative of performance of the same strains on log-based production in forest farming operations. Since we cannot evaluate all factors that may predict strain suitability, we propose that the strain that has the highest yield indoors on sawdust will display these same qualities outdoors on log-based production.

2.5.3 Storability of Mushrooms

For mushrooms as with any produce, post harvest qualities of

mushrooms are important characteristics to evaluate on new varieties. As the popularity of specialty mushrooms has increased so has consumers concern about the quality of the product. Quality of fresh mushrooms is generally judged by texture (firmness) and color (Villaescusa R, Gil MI 2002, Briones et al 1992, Zivanovic et al 2000). Fresh weight loss over time is another commonly recorded measure relating to texture (Ares et al 2006, Villaescusa 2002). A fresh weight loss of as little as 3-6% can cause marked deterioration of mushroom quality (Sveine et al 1967). While mushroom retailers commonly offer customers the choice of buying mushrooms in bulk or in plastic covered packages the national trend is towards increased sales of packaged mushrooms. One reason for this is that the mushrooms store better in terms of moisture loss and discoloration when they are packaged in a plastic container (Gromley 1969). Since respiration continues after the mushroom is picked if stored in a completely sealed plastic package the decrease in oxygen and increase in carbon dioxide concentrations can cause physiological injuries which leads to premature browning of mushrooms. If the lack of oxygen becomes severe enough to cause anaerobic respiration off odors can be a result in addition to the off color (browning) (Lopez-Briones 1992). In a study by Ares et al. (2006) mushrooms stored at atmospheric conditions experienced lower levels of deterioration compared to mushrooms stored in passive modified atmosphere packaging. Maintaining an adequate humidity while providing proper air flow can be better achieved when the mushroom product is contained in a semi permeable plastic container. Keeping the product at a cool temperature is also an important part of extending the shelf life of the mushrooms (Gromley 1975). Presumably the increased cost/value of specialty mushrooms is the reason most are sold in small prepackaged

containers (Gunn 1992).

Hericium produces white fruit bodies with high moisture content which makes bruising a common issue (Stamets 2000). The structure of the fruiting bodies, with their long thin “teeth”, also makes them prone to discoloration (browning) resulting from rapid drying during storage. For this reason retailers often ship and sell their product in the same plastic clamshell type packaging used for cherry tomatoes and berries.

Since storage and bruising are particularly challenging issues when growing *Hericium* species, storability of the mushrooms should also be examined when evaluating new mushroom strains for production. Anecdotal reports suggest that previous attempts to cultivate wild strains of *Hericium americanum* have been plagued with fruit body storage problems (pers comm Hillary Versagli head spawn maker of Phillips Mushroom Farm).

2.6 Substrate Selection

In nature, *Hericium* spp. have been known to grow on a wide variety of tree species, including red maple (*Acer rubrum)*, beech *(Fagus grandifolia)*, oak *(Quercus* spp.), walnut *(Juglans* spp), sycamore (*Platanus occidentalis)*, as well as other broadleaf trees (Stamets 2000).

Selection of tree species in forest farming mushroom production has two considerations; compatibility between mushroom species and tree species, and availability of tree species to the grower. If the idea of mushroom production in forest farming is to provide the land manager with a short term income while waiting for the timber value of the site to be realized, then species with higher timber value, like oak (*Quercus* spp.) and walnut (*Juglans* spp.), are not likely to be readily available as substrates for mushroom

production. Trees with little or no timber value such as red maple (*Acer rubrum*), poplar (*Populus* spp.) and American beech (*Fagus grandifolia*) are more likely to be removed from the forest site to make space available for the more valuable timber trees. These lesser value species are more likely to available for mushroom production.

2.7 Conclusion

Hericium mushrooms are prized edibles because of their exquisite flavor and their medicinal properties. These characteristics suggest this mushroom could compete well in the expanding specialty mushroom market. However, low availability of high yielding strains of *Hericium* that produce mushrooms that also store and ship well is one factor that prevents this mushroom from being widely available to customers at the retail level. In addition to the traditional indoor production system *Hericium* may also be a viable genus to be used in outdoor agroforestry mushroom production systems if a faster growing higher yielding strain can be discovered.

Introductions of new strains into commercial mushroom production operations often originate from wild collected specimens. A study by Uhart et al (2008) found that wild collected strains can display higher bioefficiency than the fungal strains currently being used in cultivation. There are many characteristics that should be examined when evaluating a new fungal strain for mushroom production, including growth rate, mushroom yield, bioefficiency, and productivity.

CHAPTER THREE

AN EVALUATION OF LOCAL ISOLATES OF *Hericium americanum* FOR USE IN MUSHROOM PRODUCITON

3.0 Introduction

Lion's mane mushroom (*Hericium spp*) is a common resident of decaying trees and logs throughout the Northern United States and Canada, belonging to the family Hericiaceae and the phyla Basidiomycota (Kuo 2009). All members of the genus produce white fruiting bodies (mushrooms) covered in downward cascading spines. In addition to being edible, these mushrooms have been shown to have medicinal properties (Wasser & Weiss 1999, Jeng-Leun 2001, Moldavan 2007, Wasser 2002, Hobbs 1995, Abdulla 2008) making this fungus a prime candidate for the specialty mushroom market.

Besides being grown on a large scale in indoor production farms, specialty mushrooms are also grown in agroforestry systems. Forest farming is one specific type of agroforestry in which high value non-timber specialty crops are cultivated in the unique microclimate created by a forest canopy. Specialty mushrooms can be produced utilizing logs from trees that were cut as part of a forestry management technique called thinning.

Among *Hericium* species, only *Hericium erinaceus* is currently being grown at a significant commercial level in indoor facilities. While new strains of mushroom producing fungi can be "bred" from existing strains, studies have shown that wild strains that exist in nature have the potential to out-produce isolates currently being utilized in commercial growing operations (Uhart et al 2008). The aim of this research is to determine the potential for wild collected isolates of *Hericium americanum* in commercial indoor production and in forest

farming systems. The growth rate, mushroom yield and storability of mushrooms of six locally collected isolates of *Hericium americanum* will be compared to that of a commonly available commercial strain of *Hericium erinaceus*.

3.1 Methods and Materials

General Methods and Materials

Six strains of *Hericium americanum* were collected locally from the Ithaca area in the Fall of 2007 (Table 3.1). A commercial variety of *Hericium erinaceus* was obtained from Field and Forest Products in January 2008. The cultures were maintained on Potato Dextrose Agar (PDA) (Himedia) with yeast extract (2g/L) for several months and have been stored in 10% glycerol at -80^{0}C since January 2008.

Table 3.1 Collection information of *Hericium* cultures

Hericium Spp	Culture Code	Origin/host	Date Collected
H. americanum	He 1	Ringwood Preserve, Ithaca NY on Fagus sp.	9/20/2007
H. americanum	He 2	Ringwood Preserve, Ithaca NY/ Host undetermined	10/10/2007
H. americanum	He 3	Lick Brook Preserve, Ithaca NY on Fagus sp.	10/17/2007
H. americanum	He 4	Six Mile Creek Preserve, Ithaca NY/ Host undetermined	9/16/07
H. americanum	He 5	Six Mile Creek Preserve, Ithaca NY/ Host undetermined	9/29/2007
H. americanum	He 6	Ringwood Preserve, Ithaca NY/ Host undertermined	10/10/2007
H. erinaceus	FFP3	Asian origin? Host unknown	Date unknown

Isolation was accomplished by first collecting fruiting bodies from the wild. The fruiting bodies were broken open in a laminar flow hood to expose the inner tissue and sterile forceps were then used to remove a small piece of tissue from the inner portion of the fruiting body. This piece of tissue was then placed on a Petri dish with PDA and allowed to grow at approximately 25^0C for 10 days. Three transfers were made, by taking a small section of colonized agar from the edge of the growing colony and transferring it to a new plate. If no contamination could be seen after at least three transfers the culture was considered clean and a sample of colonized agar was transferred to 10% glycerol and stored at -80^0C.

3.2 Experiment 1: Comparison of in vitro growth rates of 7 Hericium isolates at 15^0C, 25^0C, and 30^0C on PDA

According to Stamets (2000) one of the most important characteristics is to examine when evaluating a new fungal strain is speed of mycelial growth. This growth rate corresponds to the rate of substrate colonization which has direct economic importance when evaluating strains for commercial production. Rapid colonization reduces length of incubation time prior to fruiting, which results in more efficient use of production space. In addition, the substrate that remains uncolonized by mycelium remains vulnerable to mold and bacterial contaminants that could ultimately reduce yield (Zervakis 2001).

Since there is no comparative data available on the growth rate of the *Hericium* isolates collected for use in this study, their mycelial growth on PDA was compared before they were evaluated for their abilities to produce mushrooms. This portion of the study is designed to quantify the possible differences in growth rates of the six wild strains of *H. americanum* and one

commercial strain of *H. erinaceus*. Observed differences in growth rate and form were noticed early on in the isolation process. Previous studies by Ko (2005), Ginns (1985) and Imtaij (2008) showed speed of colony growth of *Hericium sp*. varied among species as well as specific isolate. Both Ko et al. (2005) and Ginn (1985) showed isolates of *Hericium americanum* had faster mycelial growth than isolates of *Hericium erinaceus.* Therefore, we hypothesize that the growth of *H. erinaceus* in culture will be less than that of the wild strains of *H. americanum*.

To quantify growth rates, the diameter of colonies growing on PDA was measured. To prepare the cultures, samples were first taken out of -80^0 C storage and allowed to grow on PDA (Himedia) at pH 6 on 90 mm plates filled with approximately 25 ml of media for 7-10 days at 25^0 C. Cubes of agar (4mm^2) were taken from the growing edge of the colonies and transferred to new identical plates with PDA (pH 6). The samples were allowed to grow in the dark at 30^0 C, 25^0 C or 15^0 C for 8 days. These temperatures were chosen based on other published experiments that show contradictory conclusions for optimum temperatures for *H. erinaceus* and *H. americanum* (Ginns 1985, Ko et al 2005, Imtiaj 2008*)*. In addition, the low temperature of 15^0 C was selected to place the cultures under a stressful temperature condition to separate out growth rate differences in case differences could not be seen under the ideal temperatures, as suggested by Fargues (1997). By testing the growth of the cultures at a wide range of temperatures, we can assure we will be able to discern differences in growth among the isolates. The diameter of the growing colonies was measured on day 4, 6 and 8. The fungal colonies tended to be circular and the average diameter of each colony was determined by averaging two measurements taken perpendicular to each other.

Measurements were taken at the same transect each time. Four replications (plates) of each strain were used at each temperature treatment and the experiment was repeated 3 times.

3.3 Experiment 2: Indoor production of Hericium mushrooms

3.3.1 General Methods

3.3.1.1 Spawn

Fungal spawn is the material that is used to inoculate the substrate on which mushrooms are to be grown. Spawn consists of fungal mycelium typically growing on sterilized grain, sawdust, wooden dowels or wood chips (Stamets 2005).

For the following experiments, fungal spawn was made in 6 liter polyethylene bags with a 4cm X 4cm micro porous breathable patch. Mixing organic rye or millet grain (59% by dry weight), gypsum ($CaSO_4$) (0.08% by dry weight), $CaCO_3$ (0.2% by dry weight) and water (40%), as described by Figlas et al. (2007). Approximately 1250g of the substrate was placed in each bag and the mixture was allowed to sit overnight (12-24 hours) to allow for the water to be absorbed by the grain. The bags were autoclaved at 121^{o}C at 15psi for 150 minutes and allowed to cool overnight before being inoculated with one strain of *H. americanum* or *H. erinaceus* the following day. This was done by adding approximately 1/4 of a 90mm plate of colonized PDA (prepared as described in the Experiment 1) to the bag. The agar piece was cut up into smaller pieces with a sterile scalpel to ensure mixing throughout the grain. The bags were sealed, shaken to mix, and allowed to colonize the grain for 2 weeks (for millet) or 3 weeks (for rye).

3.3.1.2 Growing room

A growing room measuring 5’ x 11.5’ (1.524m x 3.5052m) was used to conduct the indoor mushroom production. Two metal shelving units consisted of four shelves per unit and each shelf measured 60 cm X 120 cm. Eight sawdust bags were evenly spaced on each shelf level. A fog generator (Single head fogger MDFD-1, Plastique Frapa Inc.) with humidistat was employed to maintain the required humidity for mushroom development. An air conditioning unit was used to maintain the proper temperature. The shelves were surrounded, floor to ceiling in sheets of polyethylene to reduce air movement and aid in maintaining high humidity around the sawdust bags.

Each shelf was treated as a block due to possible differences in humidity and light levels between shelves. The bags were randomized according to isolate and sawdust type within each block. The room was set in such a way as to attempt to maintain a relative humidity of 90% and a temperature of 21^0C, as recommended by Stamets 2000, unless otherwise stated. Fluorescent lights (48” length, 32 watts) were hung above the top shelf and third shelf. Apparent light intensity, depending on location on the shelf, ranged from 500 to 1500 lux. The lights were turned on and set on a 12:12 hour light: dark cycle the same day holes were poked in the bag; before this the room was completely dark.

3.3.1.3 Sawdust bag preparation and inoculation

These experiments were initially planned to involve all six local *H. americanum* isolates and the commercial isolate of *H. erinaceus*. Due to the size of the constructed growing room and the variability found in the pilot experiment (exp 2a) the subsequent experiments were scaled down to use

only three locally collected wild *H. americanum* isolates and the one commercial strain of *H. erinaceus* each grown on two substrates, red maple and beech sawdust both supplemented with wheat bran. This allowed for 8 replications of each isolate/substrate combination. The three strains were chosen based on their performance in the agar *in vitro* growth rate study. One of the local *H. americanum* isolates with the fastest growth *in vitro*, He1, one with the slowest growth, He2, and one with a medium rate of growth, He 4, was selected to be used along with the commercial strain of *H. erinaceus*.

The moisture content of the sawdust was determined by taking fresh and oven dry weight of sawdust samples. The sawdust substrate was then prepared using the following ratios: 60% water, 31.2 % red maple or beech sawdust (d.w.), 7.8% wheat bran (d.w.), and 1% $CaSO_4$ (d.w.) (see appendix 1). Sawdust from tree species that are commonly used in the wood products industry can be obtained from a lumber mill; however beech (*Fagus grandifolia*) is not one of these species. For this reason the sawdust was created by cutting multiple “cookies” from a beech log using a chainsaw and then collecting the sawdust. Red maple (*Acer rubrum*) sawdust can be obtained from a lumber mill but because of the density difference between sawdust made in this manner and sawdust from a chain saw, the sawdust from both species was collected using a chain saw.

For these experiments the bags contained 500 g of dry sawdust, 16.03g $CaSo_4$, 125g wheat bran and 728.88g H_2O for a total weight of 1602.57g. The bags were filled with the appropriate amounts of ingredients and distilled water and then mixed by hand. The bags were autoclaved at 121^0 C for 5 hours. They were removed from the autoclave and allowed to cool overnight. Then under sterile conditions, in a laminar flow hood, each bag was inoculated with

50±1 grams of grain spawn. After inoculation each bag was sealed and shaken for three minutes per bag and then placed in the growing room. The conditions of the growing room were set as previously stated.

3.3.1.4 Mushroom Collection and Post Harvest Assessment

Four holes (appox. 3mm in diameter) were poked in each bag 10-12 days later; when mycelium could be seen colonizing the sawdust and very small primordia could be seen forming. Mushrooms began to emerge 12-14 days later. Mushrooms were collected from the bags when the branches had fully expanded and individual spines were partially formed (~5mm long). Mushrooms were collected for 5 weeks from the day the holes were poked in the bags.

For mushrooms, as with any produce, postharvest quality is an important characteristic to examine. Each collected mushroom was weighed and placed in a vented plastic clam shell container similar to that used for cherry tomatoes or berries (Specialty Fresh Food and Produce Packaging product # 13T-8). The container dimensions were 7.5 cm x 9.5 cm x 7.5 cm. The containers were then stored in a cooler set at 4 ± 1^{0}C. Each mushroom was weighed on day 3, day 6 and day 9.

A subjective quality rating was also assigned to each mushroom at each weighing (day 0, 3, 6, and 9). A subjective quality rating scale was created to quantify differences in quality between the mushrooms produced by each strain as well as determine differences in loss of quality over time. Each mushroom was given a rating value of 1 through 5. Criteria for each rating value can be found in Table 3.2. After 9 days in storage each mushroom was placed in a drying oven for at least 48 hours and the dry weight of each

mushroom was recorded.

Table 3.2 Criteria for subjective quality rating of *Hericium* mushrooms

Value	Criteria
5	Perfect mushroom Clean white color No bruising or browning Little drying of the surface Salable (0-10% of surface effected)
4	White color, could be slightly pink Minimal Bruising or Browning surface beginning to dry Salable (10-20% of surface effected)
3	Slight off white color (brown, yellow or pink), bruising or browning surface noticeably dry (20-40% of surface effected) Salable
2	Distinct off white color (could be brown yellow or pink) obvious bruising or browning surface mostly dry (40-60% of surface effected) Marginally salable
1	Worst mushroom Color is not white (yellow/ brown) Severe browning or bruising or drying > 60% of surface effected Completely unsalable

Differences in storage quality between the isolates were expected to be observed. Since *H. erinaceus* produces mushrooms with fewer branches compared with *H. americanum* it seems reasonable to assume that *H. erinaceus* would have better storage characteristics due to reduced surface to volume ratio compared to the highly branched mushrooms of *H. americanum*. Therefore, we hypothesized that the mushrooms produced by the commercial isolate, FFP3, which is H. erinaceus, will have less weight loss and higher quality ratings compared to mushrooms produced by *H americanum*.

3.3.1.5 Calculations of Yield

Mushroom yield was determined by adding the weight of all mushrooms produced per bag over the 5 week collection period. Fresh weight yield was calculated by adding the fresh weight of all the mushrooms produced per bag. Similarly, dry weight yield was calculated by adding the dry weight of all mushrooms produced per bag.

3.3.1.6 Mushroom formation inside the sawdust bag

Holes were poked in the sawdust bag to allow the mushrooms to form outside of the bag, where they can be easily harvested. However, it was not uncommon for primordia to form inside the bag rather than at the site of the hole. This can result in development of malformed mushrooms inside the sawdust bag, which would be difficult and undesirable to harvest (Illustration 3.1).

Excessive formation of malformed mushrooms inside the bag could affect the yield of collectable mushrooms that form on the outside of the bag. In an attempt to quantify the impact of this phenomenon the bags were opened after the 5 week collection period and any malformed mushrooms that had formed inside the bag were collected and weighed. The mass of malformed mushrooms was then dried for 48 hours and weighed again. We hypothesized that weight of fruiting in bag (FIB) would be negatively correlated with yield of mushrooms. In other words, the isolates that produce the most fruiting in the bag (FIB) will produce the lowest yield of harvestable mushrooms outside of the bag.

If FIB is a significant factor in determining yield of mushrooms, it may be valuable to combine the weight of yield and FIB to produce a value for total

mushroom weight produced for each bag. This can be done using fresh and dry weights to create values for total *fresh* weight of mushrooms and total *dry* weight of mushrooms produced for each bag. We hypothesize that all the isolates produce the same weight of mushrooms when the yield produced outside and inside the bag are combined. If this is true a remedy to the FIB phenomenon could cause nearly all the mushrooms to form outside the bag, thus increasing the yield of the low yielding isolates to the level of the higher yielding isolates.

Illustration 3.1 Example of fruiting in the bag phenomenon (FIB) from isolate He 2 grown on *Acer rubrum* sawdust (Ar) for exp 2d

3.3.2 Experiment 2a: Pilot experiment: Comparison of yield of 2 Hericium isolates on supplemented and unsupplemented Acer rubrum (Ar) or Fagus grandifolia (Fg) sawdust using rye grain spawn

This portion of the project was designed to get preliminary results that could then be used to make decisions about future experiments. The goal was to determine if supplementation of the sawdust substrate with wheat bran would be necessary to allow for fruiting in a timely manner and also to determine if the environmental conditions in the constructed growing room were adequate. The set up of the growing room differed from the setup as described previously in that this preliminary experiment was run prior to the addition of an air conditioning unit.

For this experiment one local isolate (He 5) and the commercial strain (FFP3) were grown on un-supplemented or supplemented red maple sawdust. The recipe for the supplemented sawdust was based on that of Joe Krawczyk of Field and Forest Products (pers. comm.). The recipe, used for the supplemented bags, consisted of red maple sawdust (*Acer rubrum*) (26% d.w.), wheat bran (13% d.w.), gypsum (0.5% d.w.) and dried molasses (0.5% d.w.) and 60% distilled water. A total of 1530g of substrate was used in each supplemented bag. Unsupplemented bags used only red maple sawdust (*Acer rubrum*) (39.5% d.w.), gypsum (0.5% d.w.) and distilled water (60%). A total of 995g of substrate was used for unsupplemented bags. After autoclaving the bags for 5 hours, the bags were allowed to cool overnight and were inoculated the following day with approximately 50g of rye grain spawn of either He 5 or FFP3. The spawn used for this experiment was created by Field and Forest Products from cultures of the two isolates we sent. The bags were placed in the growing room and the position of the bags on each shelf was randomized

by strain and +/- supplementation. Each shelf was treated as a block due to possible differences in humidity and light levels among shelves. Each of the four blocks included one replicate of each sawdust isolate combination. This randomized blocks design consisted of 2 sawdust types (tree species) X 2 supplementation treatments (with or without supplementation) X 2 isolates X 4 blocks (n=32). The temperature of the room could not be controlled at that time. A HoBo data logger recorded the temperature in the room. After 12 (for supplemented bags) or 14 (for unsupplemented bags) days 8 holes (4mm diameter) were poked in the bags and the humidity was increased to 90%. The difference in days before holes were poked was due to the fact that the mycelium in the unsupplemented bags appeared to grow much slower. Mushrooms were first harvested from the supplemented bags 15 days later. The fresh weight of the mushrooms was recorded. Dry weights of mushrooms were not recorded for this experiment. No data was collected on the FIB.

3.3.3 Experiment 2b: Comparison of yield of 4 Hericium isolates on supplemented Acer rubrum (Ar) and Fagus grandifolia (Fg) sawdust using rye grain spawn

This experiment was carried out as described previously in the general methods (3.2.1 - 3.2.1.5). Rye grain spawn was used to inoculate the sawdust bags. This was a randomized blocks design with 8 blocks x 4 isolates x 2 sawdust types x 1 replication per block (n=64). We tested the following hypotheses:

1) The growth rate of an isolate corresponds to the rate of substrate colonization which should be positively correlated with the production of

mushrooms. Thus the isolate with the fastest *in vitro* growth rate (He1) will have the highest yield.

2) Since *Hericium* mushrooms are commonly found on American beech (*Fagus grandifolia*) wood in the wild, we hypothesized that a higher fresh and dry weight yield will be found on the *Fagus grandifolia* (Fg) sawdust compared to *Acer rubrum* (Ar) sawdust.

3) Excessive formation of malformed mushrooms inside the bag (FIB) could negatively affect the yield of saleable mushrooms that form on the outside of the bag. Therefore we hypothesized is that there is an inverse relationship between FIB and yield.

4) The formation of mushrooms *inside* the sawdust bags may negatively affect the yield of collectable mushrooms that form on the outside of the bag. Some isolates may fruit more inside the bags but we hypothesized that there is no significant difference between total fresh weight yield (FIB plus externally produced mushrooms) among the isolates. The same should hold true for total dry weight yield among the isolates.

5) Mushrooms produced by *Hericium erinaceus* have less branching and a more compact appearance compared to mushrooms produced by *Hericium americanum*. For this reason *H. erinaceus* mushrooms may be less prone to drying and have better post harvest quality. We hypothesize that the mushrooms produced by the commercial isolate, FFP3 (*H. erinaceus)* will show less weight loss in storage and higher quality ratings compared to mushrooms produced by *H americanum*.

3.3.4 Experiment 2c: Comparison of yield of four Hericium isolates on supplemented Acer rubrum (Ar) and Fagus grandifolia (Fg) sawdust using millet grain spawn

This experiment was carried out as described previously in the general methods (3.2.1 - 3.2.1.5). This experiment differed from exp 2b by substituting millet grain spawn for rye grain spawn to inoculate the sawdust bags. This was a randomized blocks design with 8 blocks x 4 isolates x 2 sawdust types x 1 replication per block (n=64). Our hypotheses for this experiment are the same as those for exp 2b.

3.3.5 Experiment 2d: Comparison of yield of 4 Hericium isolates on supplemented Acer rubrum (Ar) sawdust using rye grain spawn

This experiment was carried out as described previously in the general methods (3.2.1 - 3.2.1.5)., however, only red maple (*Acer rubrum*) sawdust was used. This allowed for increased of replication; therefore two replications were present per block. Rye grain spawn was again used to inoculate the sawdust bags. This was a randomized blocks design with 8 blocks x 4 isolate types x 2 replications per block (n=64).). Our hypotheses for this experiment are the same as those for exp 2b.

3.3.6 Mushroom Production: Outdoor Totem method - Preliminary Experiment

This portion of the project was designed to explore the production capability of the local isolates when grown outdoors using an agroforestry technique. To limit the size of the experiment only three local *Hericium americanum* isolates (He 3, He 4, and He 5) and the commercial *H. erinaceus*

isolate (FFP3) were used. In March 2008, American beech (*Fagus grandifolia*) trees were felled at Cornell University's Arnot Teaching and Research Forest in Van Etten, NY and cut into 128 logs approximately 24 inches long (61 cm) with a diameter that ranged from 9-12 inches (23-31cm). The method of log inoculation used was the totem method (as described by Joe Krawczyk, Field & Forest Products, pers. comm.). The logs were cut in half into two 12 inch (30 cm) lengths. Approximately 8 oz. (237ml) of sawdust spawn was placed in the bottom of a black plastic garbage bag. Next one of the log halves was placed vertically on the 8 oz pile of sawdust. Another 8 oz. cup of sawdust spawn was placed on log and then the other corresponding half of the log was placed on its bottom half resulting in the sawdust spawn being sandwiched between the two halves. A third 8 oz. cup of spawn was placed on top of the upper portion of the log and the whole thing was covered with a large paper lawn bag. Finally, the plastic bag was pulled up over the paper bag (16" x 12" x 35") and loosely tied to conserve moisture and promote establishment of the fungi. The inoculated logs were placed on end in a laying yard, which is located under a hemlock grove at Cornell University's Arnot Teaching and Research Forest in Van Etten, NY. Since the volume of a log with a 9 inch diameter differs greatly from the volume of a log with a 12 inch diameter inoculation of the logs was randomized by log diameter. The placement of the logs in the laying yard was also randomized within block. This experiment was a randomized block design with 4 blocks x 4 *Hericium* isolates x 8 replications (n= 128).

The log inoculation took place on April 19th 2008 and the plastic bags were removed 4 months after inoculation on August 7th 2008. The paper bags were removed one year later on April 18th 2009.

Mushrooms that arose from the logs were collected when the majority

of the spines were approximately 5mm in length; the dry and fresh weight of each mushroom was recorded. The data analyzed here was collected in the fall of 2008 (Aug-Oct) and the summer of 2009 (April – July) however, the inoculated logs are expected to produce mushrooms for up to 5 years. These mushrooms will continue to be collected as before.

3.3.7 Statistical Analysis of Results

Growth rate data (exp 1) were statistically analyzed using a two way ANOVA. Growth data (mean colony diameter) were log transformed for analysis and converted back to mm for presentation in the results table. One way ANOVA models were then done by isolate and by temperature.

Fresh and dry weight yield and total mushroom weight was analyzed separately by experiment and was statistically analyzed using the ANOVA option of the JMP 2007 edition. In some cases log transformations were required to normalize data but all values were converted back (g) before being presented in tables. Block effects were controlled for by including block as a random variable in all models. Means were separated using Tukey's Range (HSD) option. Correlations between FIB and yield were analyzed using a regression model in JMP 2007.

For the post harvest data, weight loss data for exp 2b and 2c were initially combined for analysis. Next separate models were run for each substrate and the data from exp 2d were included. ANOVA models in JMP 2007 included block and experiment number as random effects. Quality data for the post harvest data were analyzed separately by experiment. The data were first analyzed using an ordinal logistics model in JMP 2007. Secondly a generalized liner model with a multinomial distribution and a cumulative logit

link function in SAS 9.2 was used to detect significant differences in the quality ratings between isolates. The variable 'block' refers to the block (shelf) in which the mushroom was grown in. The quality data from the three experiments (2b, 2c & 2d) were analyzed separately.

Data from exp 3 (outdoor production) were analyzed using a nominal logistics model in JMP 2007.

3.4 Results

3.4.1 Experiment 1: Comparison of in vitro growth rates of 7 Hericium isolates at 15^{0}C, 25^{0}C, and 30^{0}C

Isolate, temperature and the interaction between isolate and temperature were all significant in predicting colony diameter (Table 3.3). This shows while colony growth is dependent on both isolate and temperature the effect on temperature on isolate is dependent on the isolate and vice versa. Figure 3.1 shows the interaction between isolate and temperature. While the temperature increase from 15^{0}C to 25^{0}C appears to have increased the growth of all isolates the increase between 25^{0}C and 30^{0}C effects the isolates differently. Some isolates appear to have reduced growth with the temperature increase while some isolates appear to be fairly unaffected.

Table 3.3 ANOVA standard least squares using REML[1] method for colony diameter of 7 *Hericium* isolates grown on PDA at three temperatures on day 8 in experiment 1

Source	DF	F Ratio	Prob > F
Isolate	6	10.974	<0.0001**
Temperature	1	387.092	<0.0001**
Isolate x Temperature	6	4.113	0.0006*

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level. A sqrt transformation was required to normalize the data. [1] restricted maximal likelihood

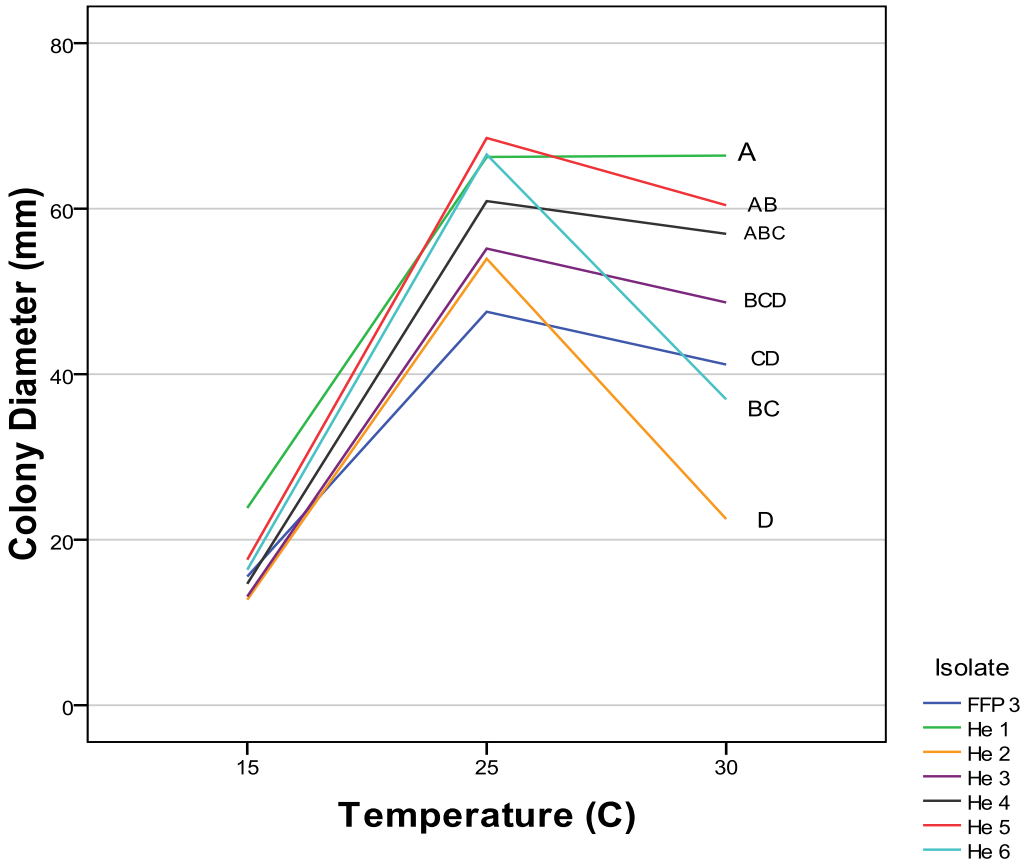

Figure 3.1 Comparison of colony diameter 7 isolates of *Hericium* isolates grown on PDA for 8 days at three temperatures (15^0C, 25^0C, 30^0C). Means not connected by the same letter are significantly different.

To further explore the effect of temperature on the mycelial growth of the isolates a one way ANOVA model was run by isolate Temperature was significant for each isolate (Table 3.4). Temperature seems to affect some isolates more than others. For example, on day 8 the mean diameter of He1 grown at 25^0C is not significantly different from the same isolate grown at 30^0C. The same is true of He 3, He 4 and He 5 cultures. Conversely, the mean diameter of FFP3, He 2 and He 6 are all significantly lower at 30^0C compared to 25^0C (Figure 3.2). This suggests that for some of the isolates 25^0C may be optimal for mycelial growth and an increase in

temperature beyond this point is detrimental to mycelial growth. At the same time, some isolates, for example He 1, appear to be more tolerant of temperature increases.

Table 3.4 ANOVA standard least squares using REML[1] method for colony diameter of *Hericium* isolates grown on PDA at three temperatures on day 8 by isolate in experiment 1

Isolate	Source	DF	F Ratio	Prob > F
FFP 3	temperature	2	536.6021	<0.0001**
He 1	temperature	2	521.6761	<0.0001**
He 2	temperature	2	252.3892	<0.0001**
He 3	temperature	2	175.5989	<0.0001**
He 4	temperature	2	152.1308	<0.0001**
He 5	temperature	2	159.9095	<0.0001**
He 6	temperature	2	145.1855	<0.0001**

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level. A log transformation was required to normalize the data [1] restricted maximal likelihood

Clear differences in the growth rate of the seven isolates across the tested temperature range (15^0C, 25^0C, 30^0C) were observed. Isolate was shown to be a significant predictor of colony diameter at each temperature (Table 3.5). The separation of means can be seen in Figure 3.3.

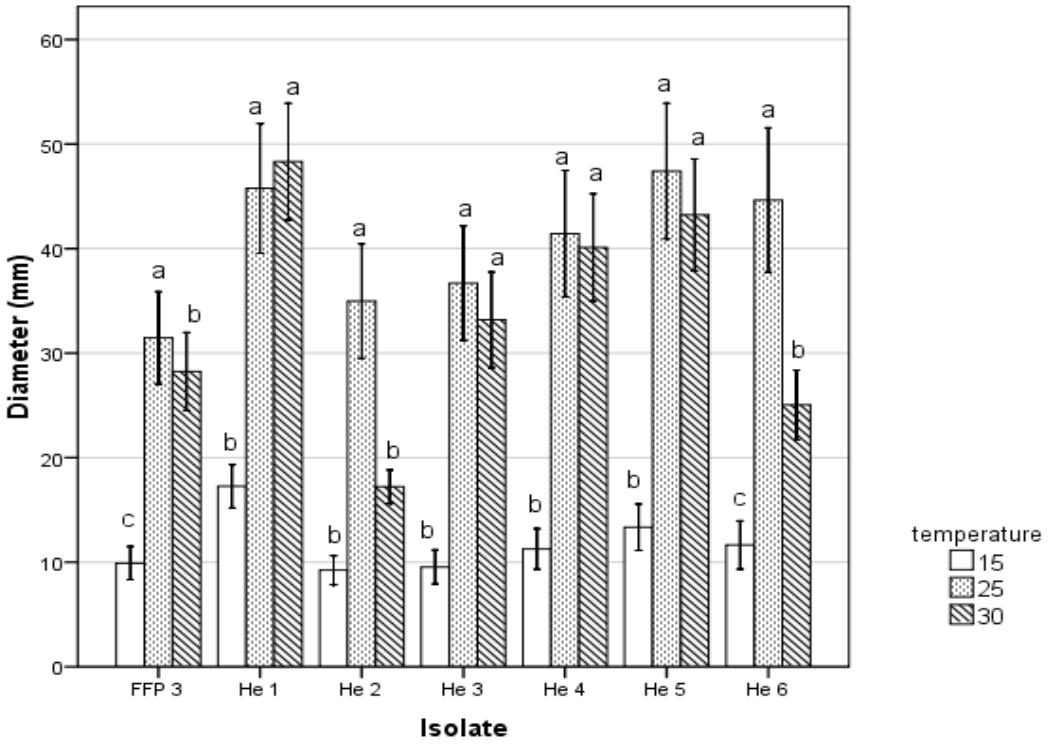

Figure 3.2 Effect of temperature on diameter (mm) of seven *Hericium* colonies grown on PDA for 8 days. *Within isolate*, values that do not share a letter are significantly ($p < 0.05$) different from one another.

Table 3.5 ANOVA standard least squares using REML[1] method for colony diameter of 7 *Hericium* isolates on day 8 grown on PDA by temperatures for Exp 1

Temperature	Source	DF	F Ratio	Prob > F
15	Isolate	6	4.3575	0.0008**
25	Isolate	6	17.6933	<0.0001**
30	Isolate	6	219.473	<0.0001**

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level A log transformation was required to normalize the data [1] restricted maximal likelihood

Isolate He 1 consistently displayed the greatest radial growth across the temperature range under investigation. Isolates He 4, He 5, and He 6 also

showed greater growth compared to the other isolates (He 2 and He 3). The commercial isolate (FFP3) of *H. erinaceus* grew less compared to most of the wild isolates. Wild isolates He 2 and He 3 also had slower growth compared to the commercial isolate. The clearest difference in growth among the isolates can be seen at 30^0C.

He 1 had greater growth compared to the other cultures at every temperature. However, He 1 was not significantly different from FFP3, He 5 and He 6 at 15^0C. At 25^0C He1, He 5, and He 6 had similar growth and at 30^0C He1 is similar to only He 5.

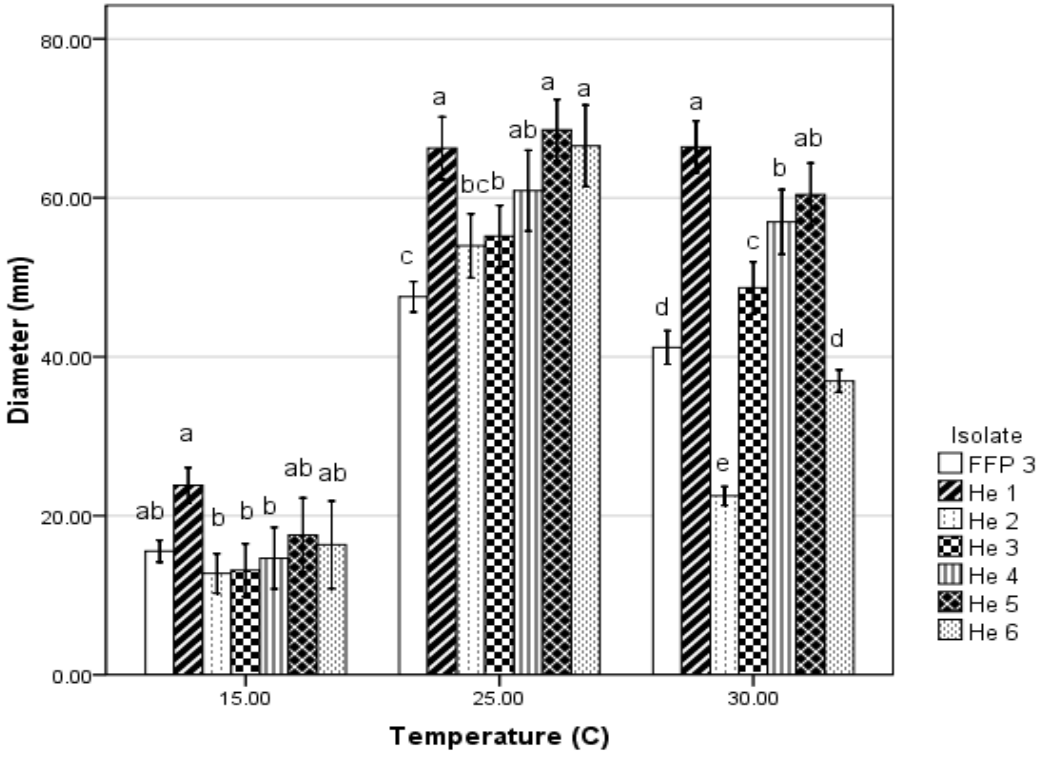

Figure 3.3 Comparison of mean diameter (mm) of seven *Hericium* colonies grown on PDA for 8 days at three temperatures (15^0C, 25^0C, 30^0C). *Within day*, values that do not share a letter are significantly ($p < 0.05$) different from one another. Error bars: +/- 2 SE

3.4.2: Results Experiment 2 Indoor mushroom production experiments

3.4.2.1 Experiment 2a: Pilot experiment: Comparison of yield of 4 Hericium isolates on supplemented and unsupplemented Acer rubrum (Ar) and Fagus grandifolia (Fg) sawdust using rye grain spawn

Very few mushrooms were collected from the unsupplemented bags. The locally collected strain (He 5) did not successfully produce any mushrooms when grown in bags of unsupplemented sawdust. The commercial strain (FFP3) did produce a very low yield of mushrooms on the unsupplemented bags (Appendix 2). We thus considered that supplementation was required for timely fruiting of the fungal strains and to ensure enough mushrooms would be produced for statistical analysis.

Among the supplemented bags, there was no significant difference ($p > 0.05$) among the fresh weights of mushrooms produced by isolate nor was there a difference in the fresh weight of mushrooms produced by substrate type (Table 3.6, Figure 3.4) . This could be due to the small sample size in this trial (n=32) and the high variability of the data.

Excessive primordia formation was observed in the bags with supplemented sawdust before holes were poked in the bag. This could have been caused by multiple factors, such as low CO_2 levels, excessive nutrients in the substrate and uneven spawn distribution. The environmental conditions in the room were also a cause for concern. Based on protocol in Stamets (2000) the optimal conditions for growing rooms were 90% humidity and a temperature of 21^0C. However, the conditions recorded by a data logger in the room showed extreme variability from these desired conditions. The relative

humidity ranged from 65-100% and the temperature fluctuated between 15^0C and 25^0C.

Table 3.6 ANOVA standard least squares mean of fresh weight yield of mushrooms produced by two *Hericium* isolates grown on one of two supplemented sawdust substrates (*Acer rubrum* or *Fagus grandifolia*) for exp 2.0 (using rye grain spawn) exp 2a

Source[1]	DF	Sum of Squares	F Ratio	Prob > F
Isolate	1	7.7187095	3.7473	0.0749
substrate	1	2.3130484	1.1230	0.3086

[1] Non-significant interactions, originally included in the model, were removed. A log-transformation was required to normalize the data.

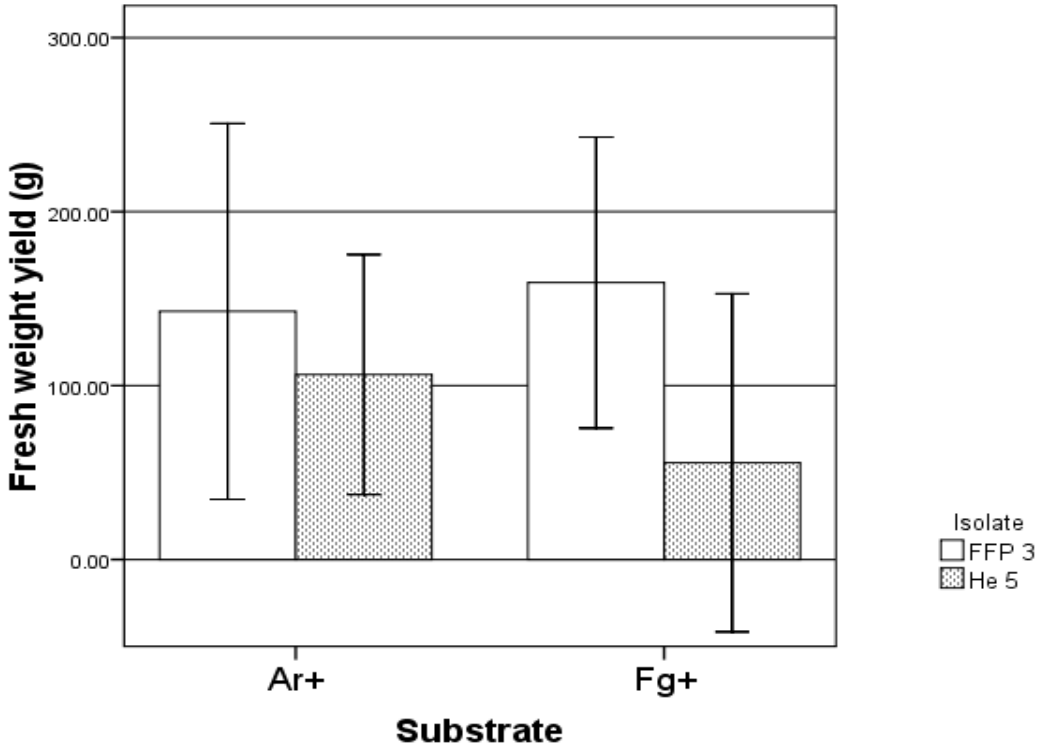

Figure 3.4 Comparison of fresh weight yield of mushrooms from two isolates of *Hericium*, a commercially grown isolate of *H. erinaceus* (FFP3) and a wild collected isolate of *H. americanum* (He 5) when grown two different substrates consisting of supplemented *Acer rubrum* sawdust (Ar+) or supplemented *Fagus grandifolia* sawdust (Fg+) for exp 2a. Error bars represent +/-2 SE

In an attempt to remedy these problems the procedures for subsequent experiments were changed in the following ways. First, dried molasses was removed from the substrate. This substance has not been reported to be used for *Hericium* spp. in a literature review of published works (Stamets 1993, Xiao 1997, Curvetto 2002, Figlas 2007, Ko 2005), or by several consulted commercial growing operations. The percent of wheat bran was reduced to 7.8% (by weight), percent moisture was reduced to 60% (by weight) and gypsum was reduced to 1% by weight. It was determined that the spawn should be m ixed more thoroughly throughout the substrate to ensure an even distribution of spawn. To aid in this effort more air space was trapped in the bag before sealing. An air conditioner was installed to maintain a more constant temperature; the polyethylene sheet also aided in maintaining humidity while the fan from the air conditioner was on.

3.4.2.2 Experiments 2b, 2c & 2d: General Results and Discussion for yield

The yield results of exp 2b, 2c, and 2d cannot statistically be compared nor can some results of the three experiments be combined and analyzed together for two reasons. First, environmental conditions in the growing room could not be controlled in such a way to maintain the consistent temperature and humidity conditions, preventing a legitimate comparison between these three experiments. The desired relative humidity and temperature conditions were 90% and 21^0C, respectively (Stamets 2005). Temperatures throughout the three experiments ranged from 16 to 25 ^{0}C. In addition, a range of 30% - 100% was recorded in the relative humidity.

The second reason results from exp 2b, 2c and 2d cannot be directly

compared or combined is because of slight differences between the methods and materials used in each experiment. Exp 2b used rye grain spawn and exp 2c used millet spawn. Exp 2d again used rye grain but differed from exp 2b by testing only one kind of sawdust, Ar (*Acer rubrum*).

3.4.2.3 Experiment 2b: Comparison of yield of 4 Hericium isolates on supplemented Acer rubrum (Ar) and Fagus grandifolia (Fg) sawdust substrate using rye grain spawn

In exp 2b, the temperature ranged from 19.5 - 25^0 C and the relative humidity ranged from 40 - 100%. Only isolate was shown to be significant in predicting fresh weight yield of mushrooms ($p<0.05$) (Table 3.7). The hypothesis that isolates grown on Fg sawdust would have significantly higher yields is rejected. The separation of means can be seen in Figure 3.5. The fresh weight yield of isolate FFP3, the commercial isolate, is significantly greater than that of the three wild collected isolates. There was no significant difference of yield among the three wild collected isolates (He1, He 2 and He 4). The hypothesis that the isolate with the fastest growth (He 1) would also have the highest fresh weight yield is rejected.

The results can also be explored by focusing on the dry weight data. Only isolate was significant ($p<0.05$) in predicting dry weight yield of mushrooms (Table 3.6). Separation of means can be seen in Figure 3.6 The dry weight yield of isolate FFP3, the commercial isolate, is significantly greater than that of the three wild collected isolates. There was no significant difference between the three wild collected isolates (He1, He 2 and He 4). Again, the hypothesis that the isolate with the fastest *in vitro* growth (He 1)

would also have the highest dry weight yield is rejected.

Table 3.7 ANOVA standard least squares using REML[1] method of fresh weight yield and dry weight yield of mushrooms produced by four *Hericium* isolates grown on wheat bran supplemented sawdust from two different tree species for exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn).

Response	Source[2]	DF	F-Ratio	Prob > F
Fresh weight yield				
	Isolate	3	10.283	<0.0001**
	Substrate	1	1.8066	0.185
Dry weight yield				
	Isolate	3	6.2847	0.0011*
	Substrate	1	0.7237	0.399

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level. [1] restricted maximal likelihood. [2] Non-significant interactions, originally included in the model, were removed.

By simply examining a graph showing FIB for each isolate, it is obvious that FIB was much less for the commercial variety of *Hericium* (FFP3) (Figure 3.5, 3.6). Only isolate was significant in predicting fresh or dry weight FIB (Table 3.8). Separation of means can be seen in Figure 3.5 and 3.6. FFP3 had significantly less of both dry and fresh weight FIB compared to any of the wild isolates.

Overall, it appears that fresh weight yield decreases as fresh weight of FIB increases (Figure 3.7 & Table 3.9). When the relationship is examined by isolate it appears that the wild isolates He 2 & He 4 shows a significant inverse relationship between fresh weight yield and FIB (Figure 3.7 & Table 3.9).

With the dry weight data it once again appears that, overall, dry weight of yield decreases as dry weight of FIB increases (Figure 3.8 & Table 3.10).

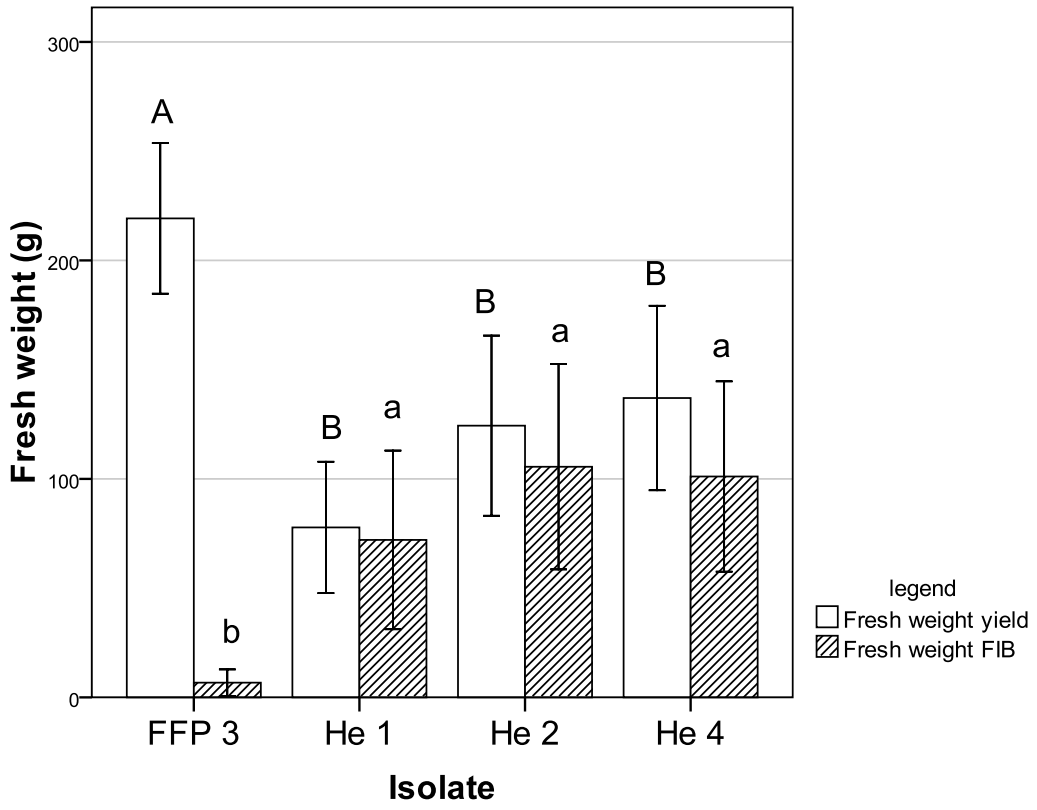

Figure 3.5 Comparison of fresh weight yield of mushrooms (g) and fresh weight of fruiting in the bag (FIB) (g) from four isolates of *Hericium*, (FFP3,a commercially grown isolate of *H. erinaceus* (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust for exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn). Means of yield not connected by the same capitalized letter are significantly different ($p < 0.05$). Means of FIB not connected by the same lowercase letter are significantly different ($p < 0.05$). Error bars represent +/- 2 SE

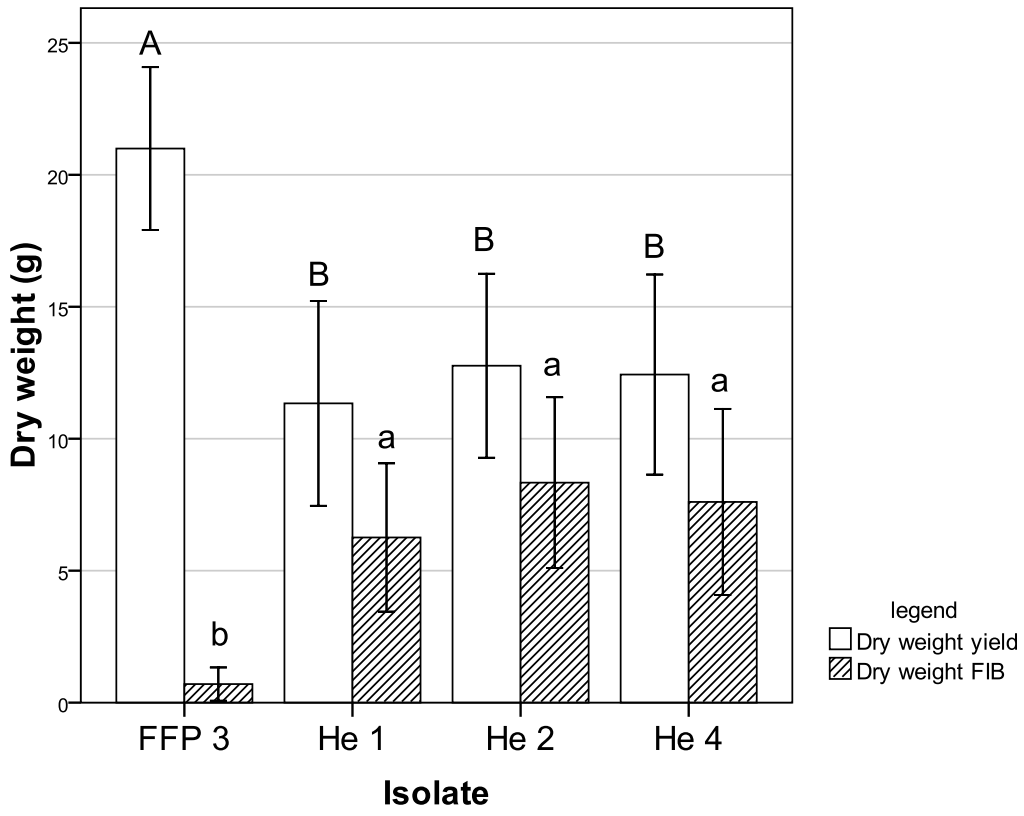

Figure 3.6 Comparison of dry weight yield of mushrooms and dry weight of fruiting in the bag (FIB) (g) from four isolates of *Hericium*, (FFP3, a commercially grown isolate of *H. erinaceus* (FFP3, a commercially grown H. erinaceus isolate and He1, He2 & He4 wild collected H. americanum isolates) grown on wheat bran supplemented sawdust for exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn). Means of yields not connected by the same capitalized letter are significantly different ($p < 0.05$).). Means of FIB not connected by the same lowercase letter are significantly different ($p < 0.05$). Error bars represent +/- 2 SE

Table 3.8 ANOVA standard least squares using REML[1] method of fresh and dry weight of fruiting inside the bag (FIB) for four isolates of *Hericium* grown on wheat bran supplemented sawdust from two tree species, *Acer rubrum* and *Fagus grandifolia* for exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn).

Response	Source[2]	DF	F-Ratio	Prob > F
Fresh weight FIB	Isolate	3	13.292	<0.0001**
	Substrate	1	0.0921	0.7626
Dry weight FIB	Isolate	3	13.0588	<0.0001**
	Substrate	1	0.0571	0.8121

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level. [1] restricted maximal likelihood . [2] Non-significant interactions, originally included in the model, were removed.

Table 3.9 Results of regression of fresh weight of yield against fresh weight of malformed mushrooms produced in the bag (FIB) for four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn)

Isolate	R^2	t-Ratio	p-value
All isolates	0.30	-5.06	<0.0001**
FFP3	0.01	-0.03	0.770
He 1	0.18	-1.75	0.101
He 2	0.36	-2.68	0.019*
He 4	0.38	-2.8	0.015*

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level

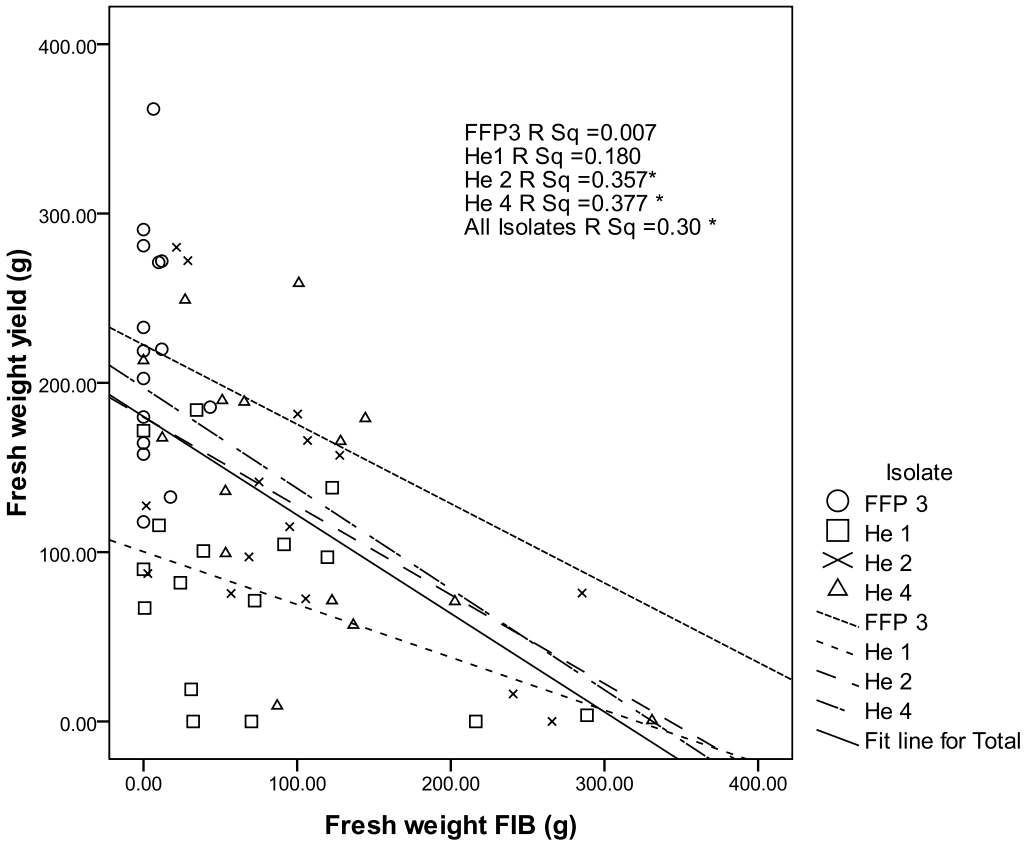

Figure 3.7 Regression of fresh weight yield of mushrooms by fresh weight of malformed mushrooms produced in the bag (FIB) produced by four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on supplemented sawdust for exp 2b (four *Hericium* isolates, two types of sawdust, rye grain).

This inverse relationship between dry weight yield and dry weight FIB seems to be stronger for the wild collected isolates (He1 R^2 = 0.20, He2 R^2 = 0.32*, He 4 R^2 = 0.44*) compared to the commercial variety (FFP3 R^2 = 0.04) (Table 3.10).

Table 3.10 Results of regression of dry weight of yield against dry weight of malformed mushrooms produced in the bag (FIB) for four Hericium isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He 4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn)

Isolate	R^2	t-Ratio	p-value
All isolates	0.37	-5.87	<0.0001**
FFP3	0.04	-0.7	0.496
He 1	0.20	-1.86	0.079
He 2	0.32	-2.51	0.026*
He 4	0.44	-3.05	0.010*

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level

For total fresh weight yield (fresh weight yield combined with fresh weight FIB), only isolate was significant ($p < 0.05$) (Table 3.11). The separation of means can be seen in Figure 3.9. Only He 1 had a significantly lower value than the commercial isolate, FFP 3, or the other two wild isolates. The hypothesis that all isolates would produce the same amount of total mushroom weight is rejected because three out of the four isolates had the same total fresh weight yield but one (He 1) had lower. However, the difference between the isolates seems minimal despite the statistical difference.

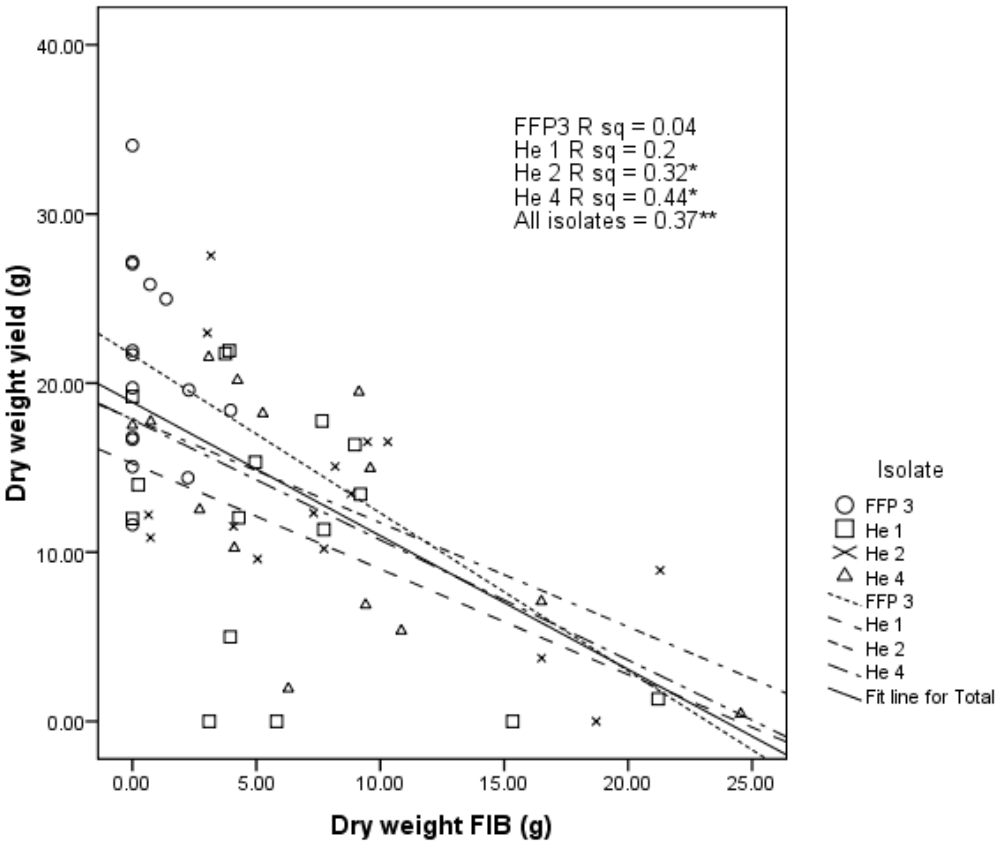

Figure 3.8 Regression of dry weight yield of mushrooms by dry weight of malformed mushrooms produced in the bag (FIB) produced by four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust for exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn).

Table 3.11 ANOVA standard least squares REML[1] method for total fresh and dry weight of mushrooms produced by four isolates of *Hericium* on two types of substrate (*Acer rubrum* or *Fagus grandifolia* sawdust) for exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn).

Response	Source[2]	DF	F-Ratio	Prob > F
Total fresh weight yield				
	Isolate	3	4.8328	0.0046*
	Substrate	1	0.8902	0.3495
Total dry weight yield				
	Isolate	3	1.4224	0.2475
	Substrate	1	0.8586	0.3587

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level. [1] restricted maximal likelihood. [2] Non-significant interactions, originally included in the model, were removed. [1] Non-significant interactions, originally included in model, were removed. A log transformation was used to normalize the data.

For total dry weight yield (dry weight yield combined with dry FIB), neither isolate nor substrate was significant (Table 3.11). There is no difference between the means of dry total mushroom weight (Figure 3.10). In this case the hypothesis cannot be rejected; there is no difference in total weight of mushrooms among all isolates. The data for total dry weight has a similar tendency as that of total fresh weight data. It appears that the difference among isolates for total fresh mushroom weight is only due to differences in moisture content in the mushrooms. He 1 was found to have lower fresh weight yield than the other three isolates but when the dry weights are compared no difference can be found among the isolates. The difference in total fresh weight yield therefore reflects only a difference in water content among the yields of the isolates.

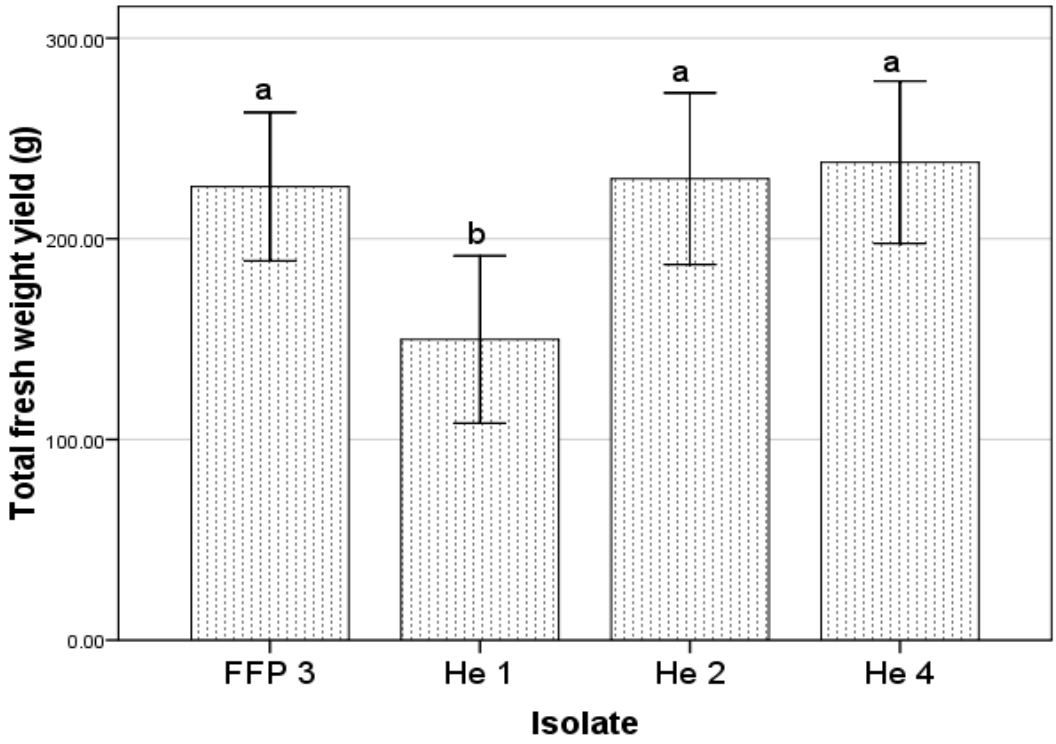

Figure 3.9 Comparison of total fresh weight (g) of mushrooms (fresh weight yield combined with fresh weight of fruiting inside the bag (FIB)) by four *Hericium* isolates (FFP3, a commercially grown H. erinaceus isolate and He1, He2 & He4 wild collected H. americanum isolates) grown on supplemented sawdust (*Acer rubrum* or *Fagus grandifolia*) for exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn). Means not connected by the same letter are significantly different ($p < 0.05$). Error bars: +/- 2 SE

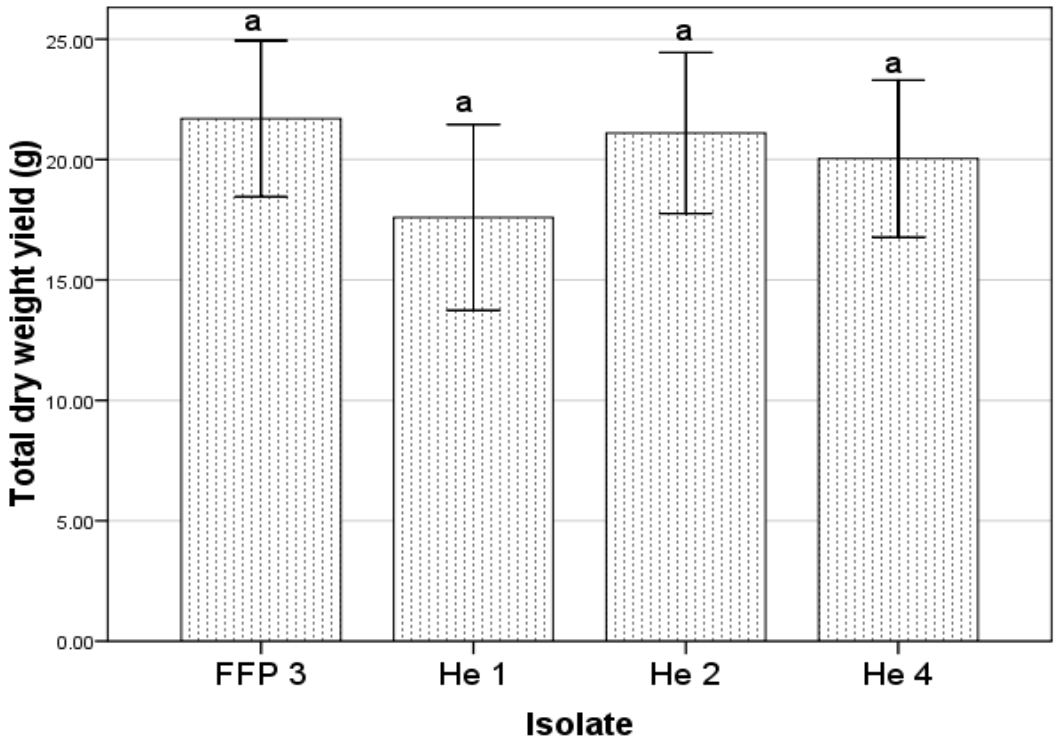

Figure 3.10 Comparison of total dry weight (g) of mushrooms (dry weight yield combined with dry weight of malformed mushrooms produced inside the bag (FIB)) by four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on supplemented sawdust (*Acer rubrum* or *Fagus grandifolia*) for exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn). Means not connected by the same letter are significantly different ($p < 0.05$). Error bars: +/- 2 SE

3.4.2.4 Experiment 2c: Comparison of yield of 4 Hericium isolates on supplemented Acer rubrum (Ar) and Fagus grandifolia (Fg) sawdust using millet grain spawn

This experiment differed from exp 2b in that millet grain spawn was used. During the experiment temperature ranged from 16.5 – 23.5^0 C and the relative humidity ranged from 30 – 100%. Compared to the data collected from exp 2b the overall mean yield was lower and many of the bags did not produce any mushrooms at all. If the graphs from experiment 2b (Figure 3.5 & 3.6) are

compared to those of experiment 2c (Figure 3.11 to Figure 3.12) the proportions of yield and FIB appear to be reversed. Weight of FIB exceeds weight of yield in experiment 2c while the opposite is true for experiment 2b.

When fresh weight yield was examined only isolate was significant ($p < 0.05$) (Table 3.12). The separation of means can be seen in Figure 3.11. The hypothesis that isolates grown on Fg sawdust would have significantly higher yields is rejected. The hypothesis that the isolate that demonstrated the fastest growth *in vitro* (He 1) would have the highest yield is also rejected. There is no significant difference between fresh weight yield of the commercial isolate (FFP3) and one of the wild isolates, He 4.

Table 3.12 ANOVA standard least squares using REML[1] method of fresh weight yield and dry weight yield of mushrooms produced by four *Hericium* isolates grown on wheat bran supplemented sawdust from two different tree species (*Fagus grandifolia* or *Acer rubrum*) for exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn).

Response	Source[2]	DF	F Ratio	Prob > F
Fresh weight yield				
	Isolate	3	5.9814	0.0014*
	Substrate	1	0.0101	0.9204
Dry weight yield				
	Isolate	3	2.1095	0.1102
	Substrate	1	0.0006	0.9804

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level. [1] restricted maximal likelihood. [2] Non-significant interactions, originally included in the model, were removed. A log-transformation was required to normalize the data.

For dry weight yield neither isolate nor substrate was significant ($p < 0.05$) (Table 3.12 & Figure 3.12). The hypothesis that isolates grown on Fg sawdust would have significantly higher yields is rejected.

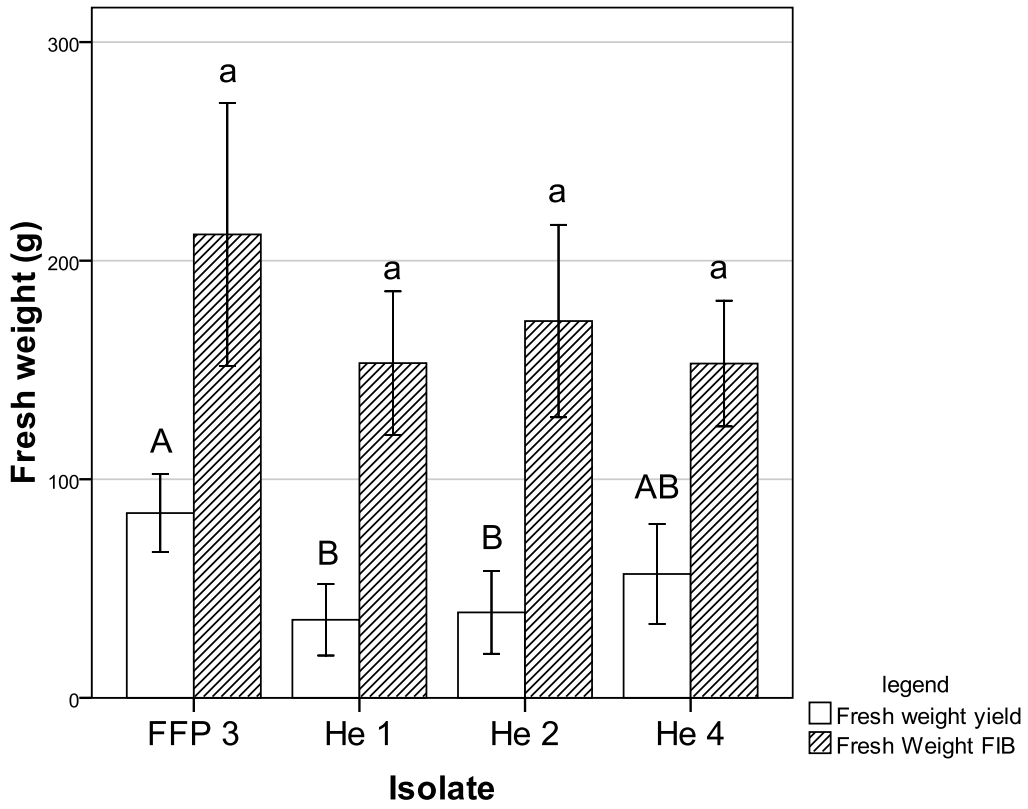

Figure 3.11 Comparison of fresh weight yield of mushrooms and fresh weight of fruiting inside the bag (FIB) for four isolates of *Hericium* (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust for exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn). Yield means not connected by the same capitalized letter are significantly different ($p < 0.05$). FIB means not connected by the same lowercase letter are significantly different ($p < 0.05$). Error bars represent +/- 2 SE

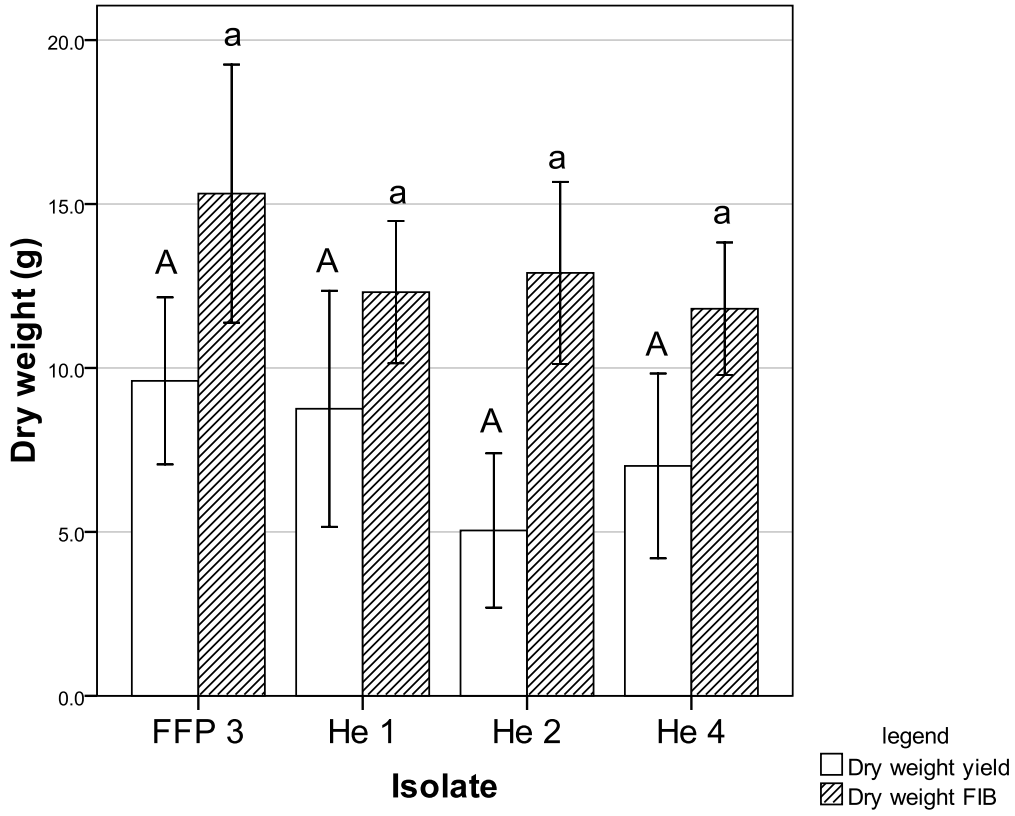

Figure 3.12 Comparison of dry weight yield of mushrooms and weight of fruiting inside the bag (FIB) for four isolates of *Hericium*, (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on supplemented sawdust for exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn). Yield means not connected by the same capitalized letter are significantly different ($p < 0.05$). FIB means not connected by the same lowercase letter are significantly different ($p < 0.05$). Error bars represent +/- 2 SE

Also the hypothesis that the isolate with the fastest *in vitro* growth would have the greatest dry weight yield is also rejected. There was no difference among the dry weight yields of all the isolates.

To explore the phenomenon of FIB ANOVA models were run. A log transformation normalizes the dry and fresh weight FIB data. Only substrate was significant ($p<0.05$) (Table 3.13) in predicting fresh weight FIB, with Fg (*Fagus grandifolia*) producing greater FIB compared to *Acer rubrum* (Ar) sawdust (Figure 3.13). However, the difference between the substrates seems minimal despite the statistical difference. There was no statistical difference between the fresh weight FIB of the isolates (Figure 3.11).

With dry weight FIB, isolate was not found to be significant and substrate was marginally not significant ($p=0.096$) (Table 3.13). It appears that substrate may have something to do with the amount of FIB, which in turn appears to affects the yield of the bag.

Table 3.13 ANOVA standard least squares using REML[1] method of fresh and dry weight of malformed mushroom produced inside the bag (FIB) for four isolates of *Hericium* grown on supplemented sawdust from two tree species, *Acer rubrum* and *Fagus grandifolia* for exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn)

Response	Source[2]	DF	F-Ratio	Prob > F
Fresh weight FIB				
	Isolate	3	0.8731	0.4611
	Substrate	1	5.4122	0.0239*
Dry weight FIB				
	Isolate	3	0.5317	0.6626
	Substrate	1	2.8768	0.0958

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level. [1] restricted maximal likelihood. [2] Non-significant interactions, originally included in the model, were removed. A log-transformation was required to normalize the data.

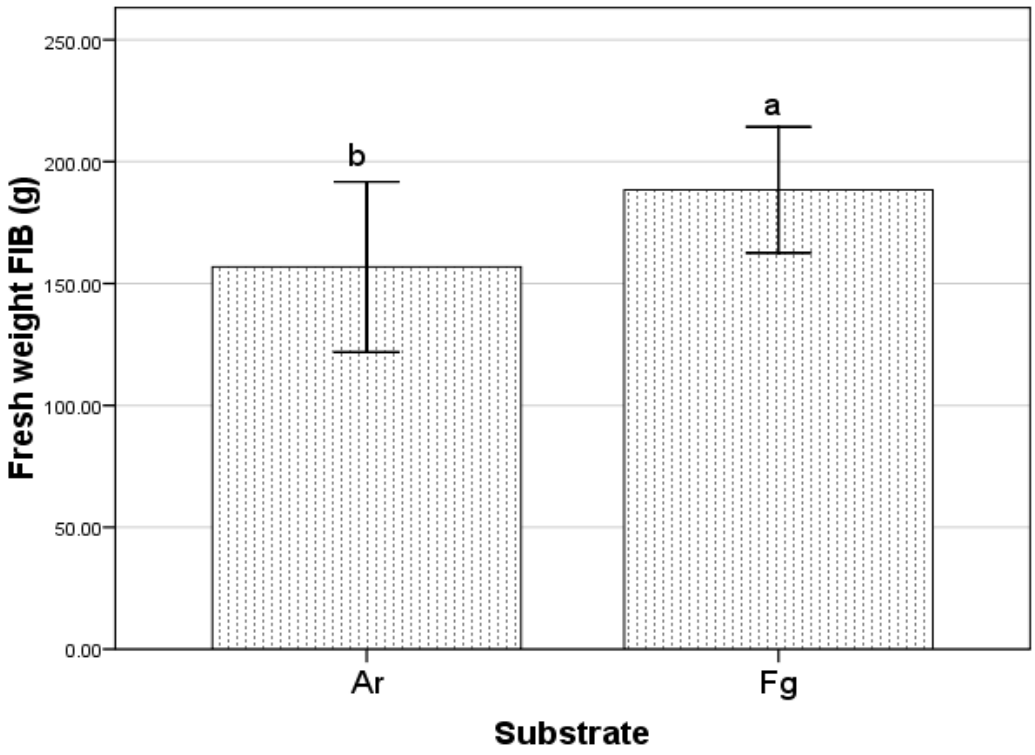

Figure 3.13 Comparison of fresh weight of fruiting inside the bag (FIB) of Hericium isolates when grown on wheat bran supplemented *Acer rubrum* (Ar) or *Fagus grandifolia* (Fg) sawdust for exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn). Error bars: +/- 2 SE

Since substrate was found to be significant in predicting fresh weight FIB in this experiment, separate regression models of fresh weight yield against fresh weight FIB were run for each sawdust type. There was no significant relationship found between fresh weight yield and fresh weight FIB (Table 3.14 & Figure 3.14). For the dry weight data, only He 1 when grown Fg sawdust showed a significant relationship between dry weight yield and dry weight FIB (Table 3.15 & Figure 3.15). We hypothesized that there is an inverse relationship between yield and FIB and overall, it was rejected for both fresh and dry weight data when inoculated with millet grain spawn.

Table 3.14 Results of regression of fresh weight of yield against fresh weight of malformed mushrooms produced in the bag (FIB) for four Hericium isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He 4, wild collected *H. americanum* isolates) grown on two substrates, wheat bran supplemented sawdust of *Acer rubrum* (Ar) or *Fagus grandifolia (*Fg*)* exp 2b (four *Hericium* isolates, two sawdust types, millet grain spawn)

Substrate	Isolate	R^2	t-ratio	p-value
Ar				
	All isolates	0.002	0.25	0.805
	FFP3	0.044	-0.53	0.616
	He 1	0.005	-0.18	0.863
	He 2	0.021	0.36	0.732
	He 4	0.021	0.36	0.733
Fg				
	All isolates	0.000	0.02	0.983
	FFP3	0.075	0.70	0.512
	He 1	0.371	-1.88	0.109
	He 2	0.158	-1.06	0.330
	He 4	0.218	1.29	0.244

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level

The total fresh weight data (fresh weight yield combined with fresh weight FIB) was not normal and required a log transformation. Only substrate was significant ($p < 0.05$) (Table 3.16). Bags with Fg sawdust produced significantly more total fresh weight as compared to Ar (Figure 3.16). Again while this difference was found to be statistically significantly different the actual difference appears to be minimal. To further explore this data models were run separately for each substrate. In both cases, isolate was not significant and the means are not significantly different (Table 3.17). The hypothesis that all total fresh weight means will be equal cannot be rejected (Figure 3.17).

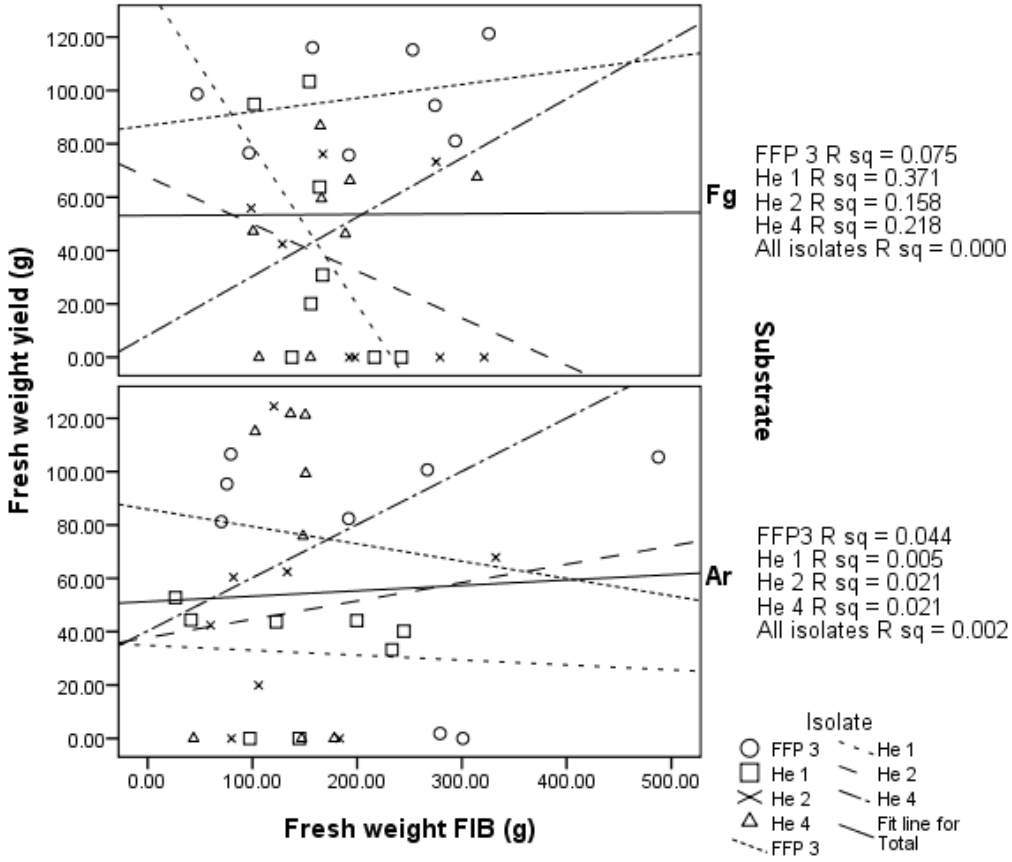

Figure 3.14 Regression of fresh weight yield of mushrooms by fresh weight of malformed mushrooms produced in the bag (FIB) by four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on two substrates (wheat bran supplemented sawdust of *Acer rubrum* (Ar) or *Fagus grandifolia* (Fg) for exp 2c (four *Hericium* isolates, two types of sawdust, millet grain spawn).

Table 3.15 Results of regression of dry weight of yield against dry weight of malformed mushrooms produced in the bag (FIB) for four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and three wild collected *H. americanum* isolates He1, He2 & He 4) grown on two substrates, wheat bran supplemented sawdust of *Acer rubrum* (Ar) or *Fagus grandifolia (*Fg*)* exp 2b (four *Hericium* isolates, two sawdust types, millet grain spawn)

Substrate	Isolate	R^2	t-ratio	p-value
Ar				
	All isolates	0.06	1.43	0.164
	FFP3	0.048	0.55	0.601
	He 1	0.145	1.01	0.35
	He 2	0.098	0.81	0.451
	He 4	0.075	0.70	0.513
Fg				
	All isolates	0.005	-0.42	0.677
	FFP3	0.228	1.33	0.231
	He 1	0.691	-3.66	0.011*
	He 2	0.066	-0.65	0.538
	He 4	0.281	1.53	0.177

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level

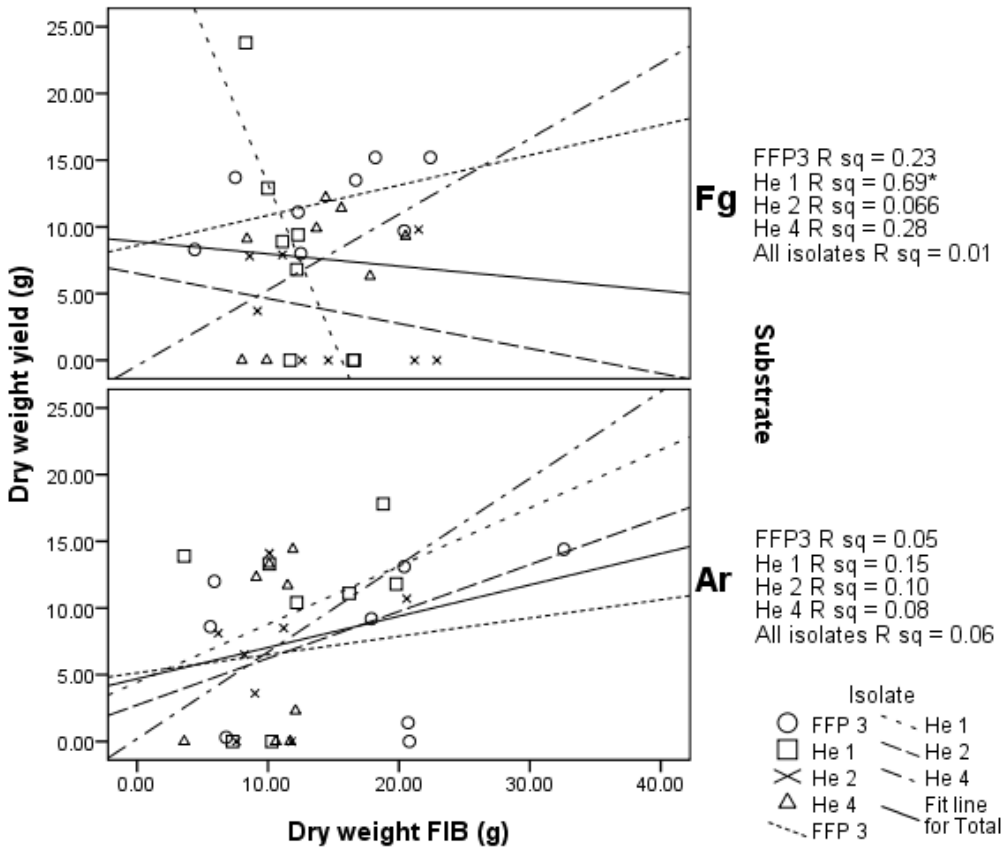

Figure 3.15 Regression of dry weight yield of mushrooms by dry weight of malformed mushrooms produced in the bag (FIB) by four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on two substrates (wheat bran supplemented sawdust of *Acer rubrum* (Ar) or *Fagus grandifolia* (Fg)) for exp 2c (four Hericium isolates, two types of sawdust, millet grain spawn).

Table 3.16 ANOVA standard least squares using REML[1] method for total fresh and dry weight of mushrooms produced by four isolates of *Hericium* on two types of substrate (*Acer rubrum* or *Fagus grandifolia* sawdust) for exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn)

Response	Source[2]	DF	F-Ratio	Prob > F
Total fresh weight				
	Isolate	3	0.9254	0.4351
	Substrate	1	5.4249	0.0238*
Total dry weight				
	Isolate	3	2.453	0.0736
	Substrate	1	0.6145	0.4367

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level. [1] restricted maximal likelihood. [2] Non-significant interactions, originally included in model, were removed. A log transformation was used to normalize the data.

Table 3.17 ANOVA standard least squares using REML[1] method for total fresh weight of mushrooms produced by four isolates of *Hericium* on two types of substrate ,*Acer rubrum* (Ar) and *Fagus grandifolia* (Fg) sawdust for exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn)

Substrate	Source	DF	F-Ratio	Prob > F
Ar				
	Isolate	3	1.9954	0.1455
Fg				
	Isolate	3	1.0847	0.3772

[1] restricted maximal likelihood

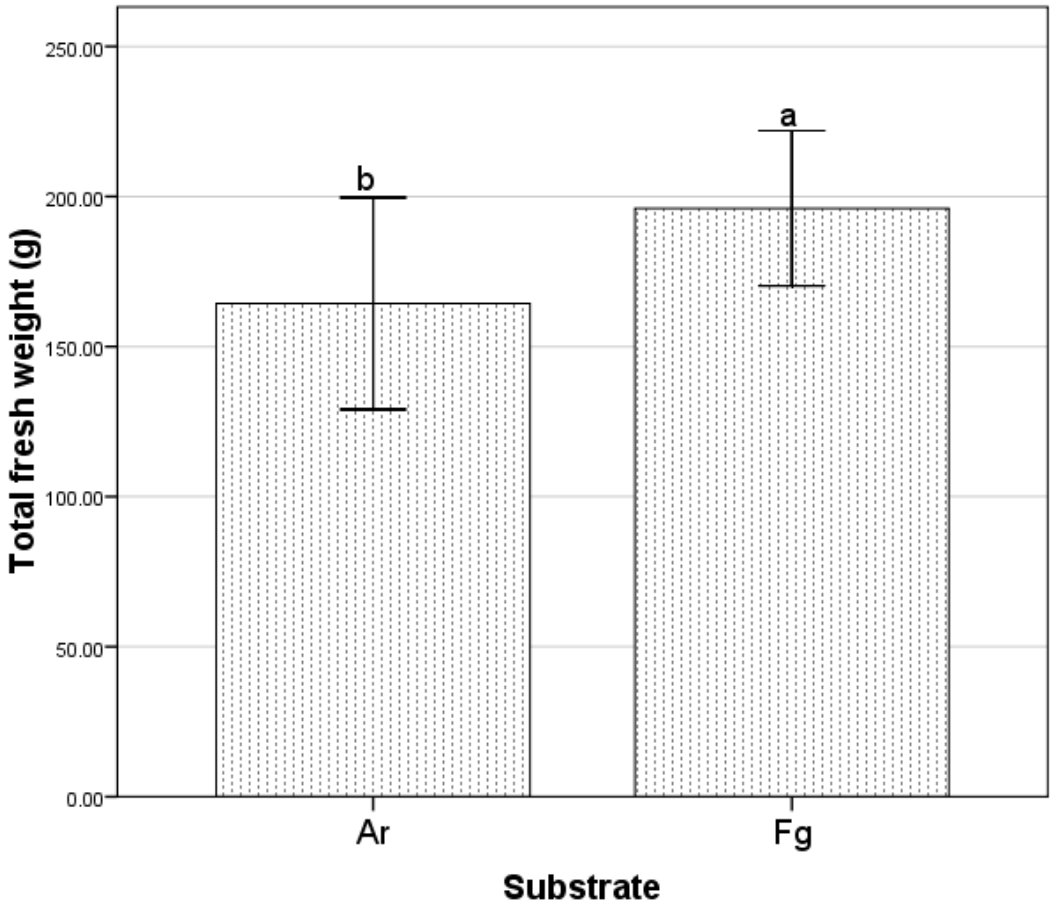

Figure 3.16 Comparison of total fresh weight of mushrooms (fresh weight yield combined with fresh weight of mushrooms produced in the bag (FIB)) for *Hericium* isolates grown on wheat bran supplemented sawdust from *Fagus grandifolia* (Fg) or *Acer rubrum* (Ar) exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn). Levels not connected by the same letter are significantly different ($p < 0.05$). Error bars: +/- 2 SE

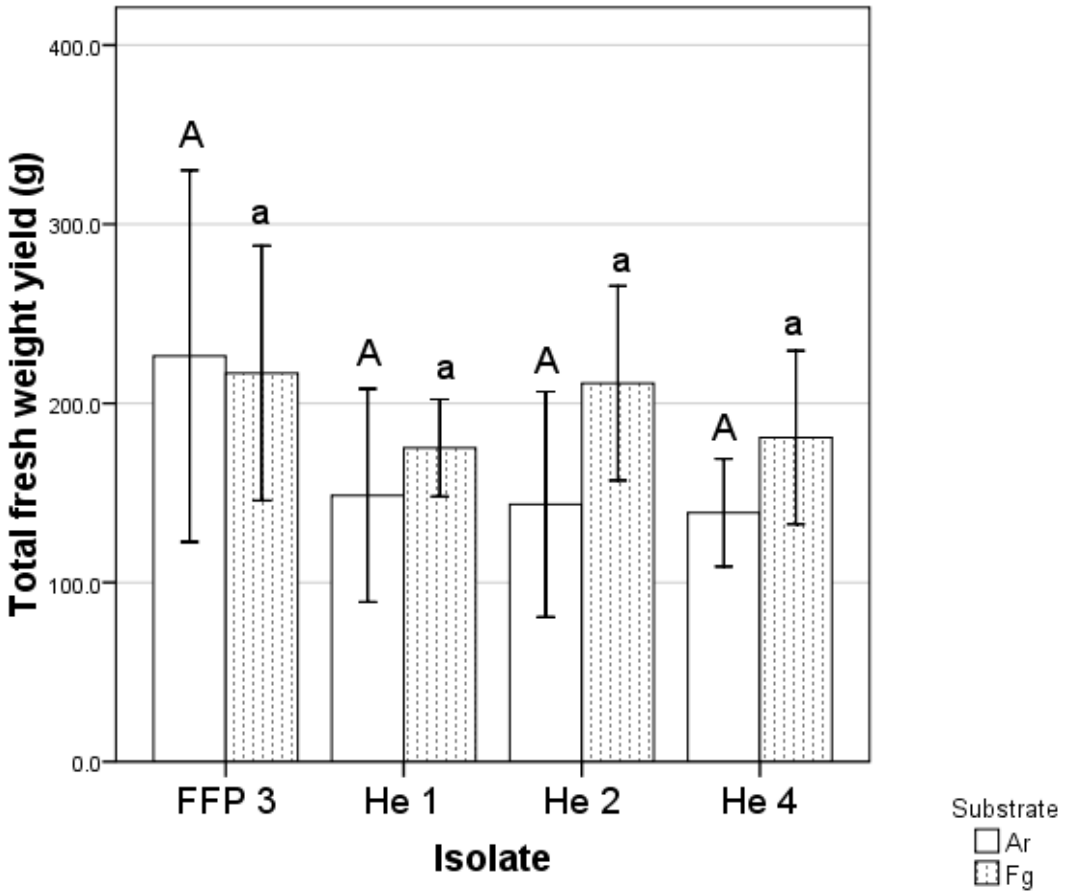

Figure 3.17 Comparison of total fresh weight of mushrooms (fresh weight yield combined with fresh weight of fruiting in the bag (FIB)) for four *Hericium* isolates (FFP3, a commercially grown H. erinaceus isolate and He1, He2 & He4 wild collected H. americanum isolates) grown on two substrates, wheat bran supplemented sawdust from *Acer rubrum* (Ar) or *Fagus grandifolia* (Fg) exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn). Levels not connected by the same capitalized letter are significantly different ($p < 0.05$). Levels not connected by the same lowercase letter are significantly different ($p < 0.05$). Error bars: +/- 2 SE

The total dry weight data (dry weight yield combined with dry weight FIB) was normal. Substrate was not significant and isolate was marginally not significant (p=0.074) (Table 3.16). So again the means cannot be separated by isolate (Figure 3.18). The hypothesis that all total dry weight means will be equal is accepted.

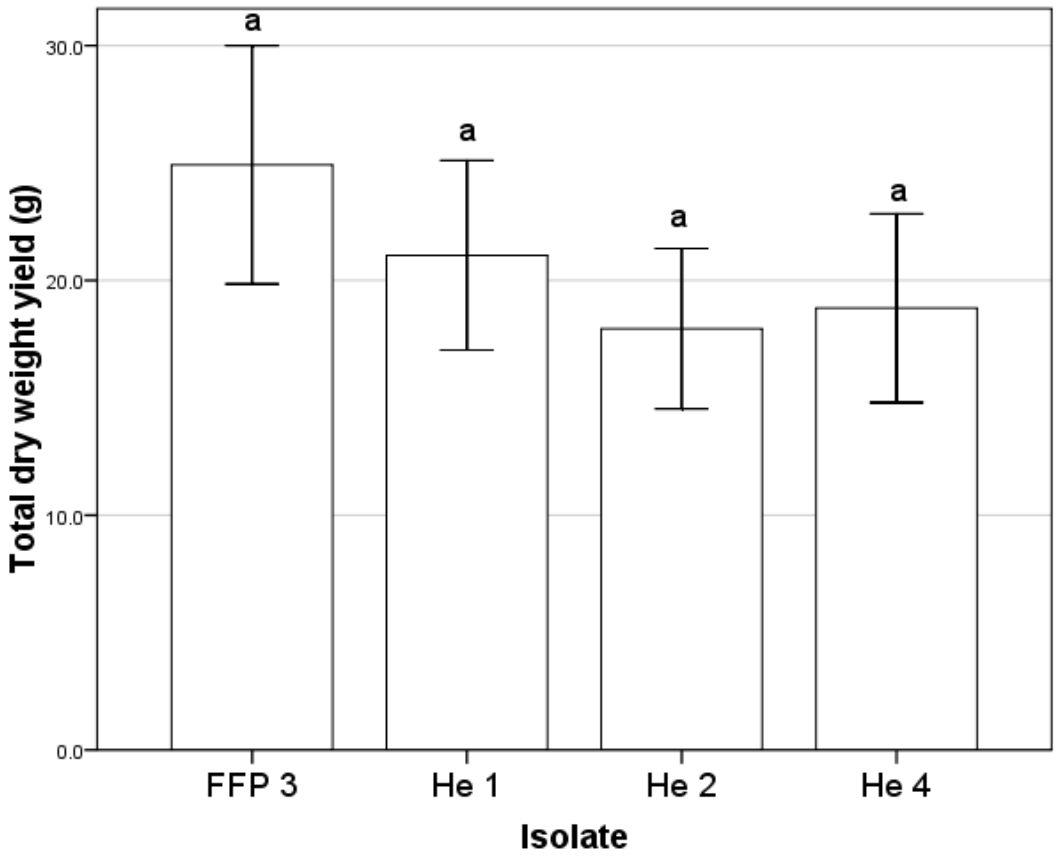

Figure 3.18 Comparison of total dry weight (dry weight yield combined with dry weight of fruiting in the bag (FIB)) for four *Hericium* isolates (FFP3, a commercially grown H. erinaceus isolate and He1, He2 & He 4 wild collected H. americanum isolates) grown on wheat bran supplemented sawdust from *Acer rubrum* or *Fagus grandifolia* exp 2c (four *Hericium* isolates, two sawdust types, using millet grain spawn). Levels not connected by the same letter are significantly different ($p < 0.05$). Error bars: +/- 2 SE

3.4.2.5 Experiment 2d: Comparison of yield of 4 Hericium isolates on supplemented Acer rubrum (Ar) sawdust using rye grain spawn

This experiment used rye grain spawn and only one variety of sawdust, Ar (*Acer rubrum*). During this experiment the temperature ranged from 18 – 22^0 C and the relative humidity ranged from 40 – 100%. Isolate was significant in predicting fresh or dry weight yield of mushrooms ($p< 0.05$) (Table 3.18). The separation of means can be seen in Figure 3.19 and 3.20. The hypothesis

that the isolate with the fastest growth *in vitro* (He 1) will have the greatest yield is rejected.

Table 3.18 ANOVA standard least squares using REML[1] method of fresh weight yield and dry weight yield of mushrooms produced by four *Hericium* isolates grown on supplemented sawdust for exp 2d (four *Hericium* isolates, one sawdust type, using rye grain spawn).

Response	Source[2]	DF	F-Ratio	Prob < F
Fresh weight yield				
	Isolate	3	10.61	<0.0001**
Dry weight yield				
	Isolate	3	9.4507	<0.0001**

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level. [1] restricted maximal likelihood. [1] Non-significant interactions, originally included in the model, were removed.

Isolate was significant in predicting both dry and fresh weight FIB ($p< 0.05$) (Table 3.19). In both cases, FIB of FFP3 was significantly less than that of the other isolates (Figure 3.20 & 3.21).

A regression of fresh weight yield by fresh weight of FIB shows a strong relationship (R=0.73*) (Table 3.20) When the same regression is done for each isolate a strong correlation is found with all the wild collected isolates (He 1 R^2=0.75**, He 2 R^2= 0.80**, He 4 R^2 = 0.89**), but not the commercial isolate, FFP3 (FFP3 R^2 = 0.03) (Table 3.20 & Figure 3.21).

A regression of dry weight by dry weight FIB again showed a strong correlation over all with a R^2 value of 0.76 (Table 3.21). When the regression is separated by isolate, a strong relationship can again be seen for the wild collected isolates (He 1, R^2 = 0.91**, He 2 R^2 = 0.81**, He 4 R^2 = 0.91**)

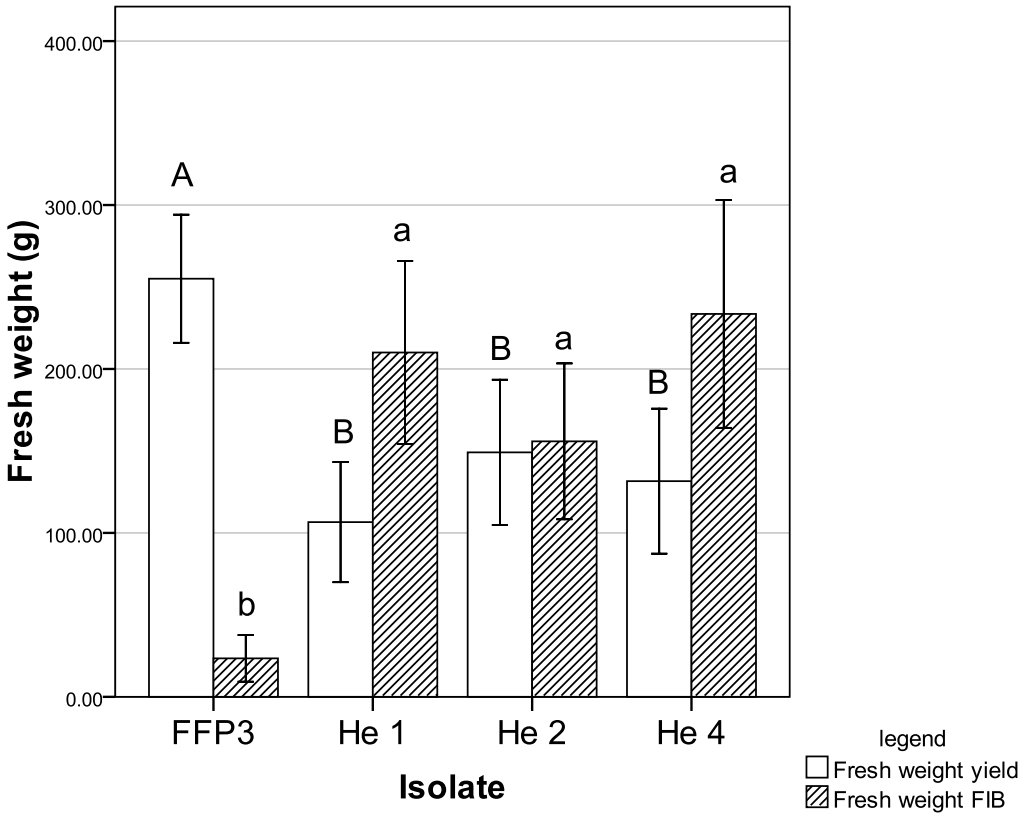

Figure 3.19 Comparison of fresh weight yield (g) and fresh weight of fruiting inside the bag (FIB) (g) of four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust for exp 2d (four *Hericium* isolates, one sawdust type, using rye grain spawn). Levels not connected by the same capitalized letter are significantly different ($p < 0.05$). Levels not connected by the same lowercase letter are significantly different ($p < 0.05$). Error bars: +/- 2 SE

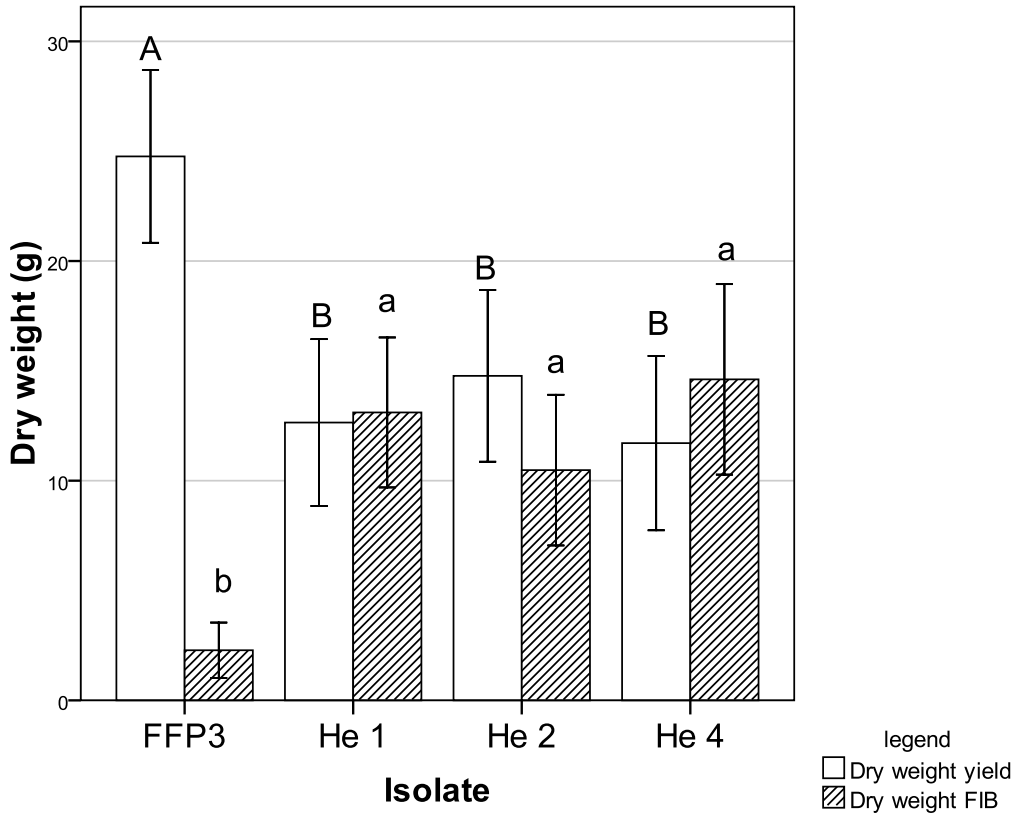

Figure 3.20 Comparison of fresh weight yield (g) and dry weight of fruiting inside the bag (FIB) (g) of four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust for exp 2d (four *Hericium* isolates, one sawdust type, using rye grain spawn). Levels not connected by the same letter are significantly different ($p < 0.05$). Error bars: +/- 2 SE

Table 3.19 ANOVA standard least squares using REML[1] method of fresh and dry weight of malformed mushroom produced inside the bag (FIB) for four isolates of *Hericium* grown on wheat bran supplemented sawdust from *Acer rubrum* for exp2d (four *Hericium* isolates, one sawdust type, rye grain sawdust.

Response	Source	DF	F Ratio	Prob > F
Fresh weight FIB				
	Isolate	3	26.6488	<0.0001**
Dry weight FIB				
	Isolate	3	20.0544	<0.0001**

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level. [1] restricted maximal likelihood

Table 3.20 Results of regression of fresh weight of mushrooms against fresh weight of malformed mushrooms produced in the bag (FIB) for four FFP3, a commercially grown *H. erinaceus* isolate and He 1, He 2 & He 4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust exp 2d (four *Hericium* isolates, one sawdust type, rye grain spawn)

Isolate	R^2	t-Ratio	p-value
All Isolates	0.73	-12.84	<0.0001**
FFP3	0.03	-0.64	0.54
He 1	0.75	-6.44	<0.0001**
He 2	0.80	-7.29	<0.0001**
He 4	0.89	-10.54	<0.0001**

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level.

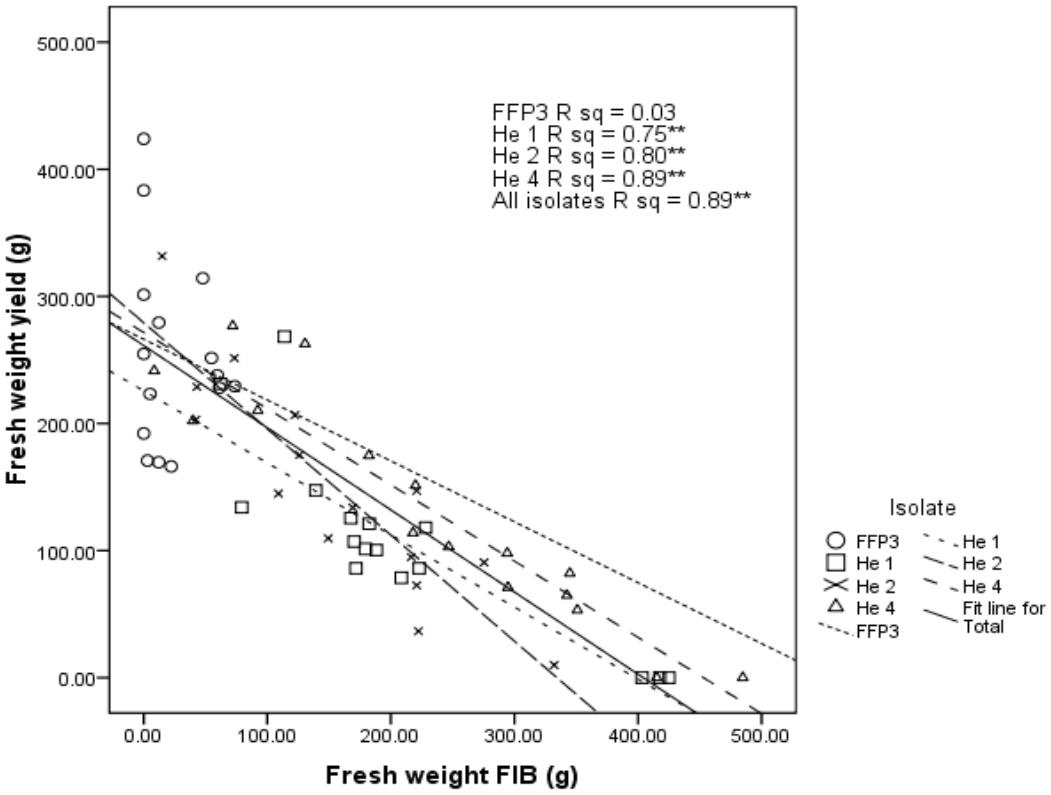

Figure 3.21 Regression of fresh weight yield of mushrooms by fresh weight of malformed mushrooms produced in the bag (FIB) produced by four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust for exp 2d (four *Hericium* isolates, one sawdust type, using rye grain spawn).

compared to the relationship between the commercial isolate (FFP3 0.02) (Table 3.21 & Figure 3.22). This shows that only the wild isolates show a strong inverse relationship between dry weight yield and dry weight FIB. The hypothesis that there is an inverse relationship between yield and FIB is supported by both the fresh and dry weight data for the wild isolates.

When examining the total fresh weight yield, isolate was significant (Table 3.22). The separation of means can be seen in Figure 3.23. It appears that He 4 and He1 had the highest total fresh mushroom weight, followed closely behind by He 2 and FFP3.

Table 3.21 Results of regression of dry weight of mushrooms against dry weight of malformed mushrooms produced in the bag (FIB) for four FFP3, a commercially grown *H. erinaceus* isolate and He1, He 2 & He 4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust exp 2d (four *Hericium* isolates, one sawdust type, rye grain spawn)

Isolate	R^2	t-Ratio	Prob > ItI
All	0.76	-13.89	<0.0001**
FFP3	0.023	-0.63	0.54
He 1	0.91	-11.77	<0.0001**
He 2	0.81	-7.78	<0.0001**
He 4	0.91	-12.06	<0.0001**

*denotes significance at p = 0.05 level **denotes significance at p = 0.001 level.

The total dry weight data was not normal and required a log transformation. Isolate was not significant (p > 0.05) and the means of the isolates cannot be separated (Table 3.22) (Figure 3.24). Again, this supports

the hypothesis that there is no difference between the total dry weights of the isolates.

These results are similar to what was found in exp 2b. Statistical differences were found among the total fresh weight yields of the isolates but no difference was found among the total dry weight yields of the isolates. This again shows that the difference in fresh weight yield must be based solely on differences in moisture content of the mushrooms.

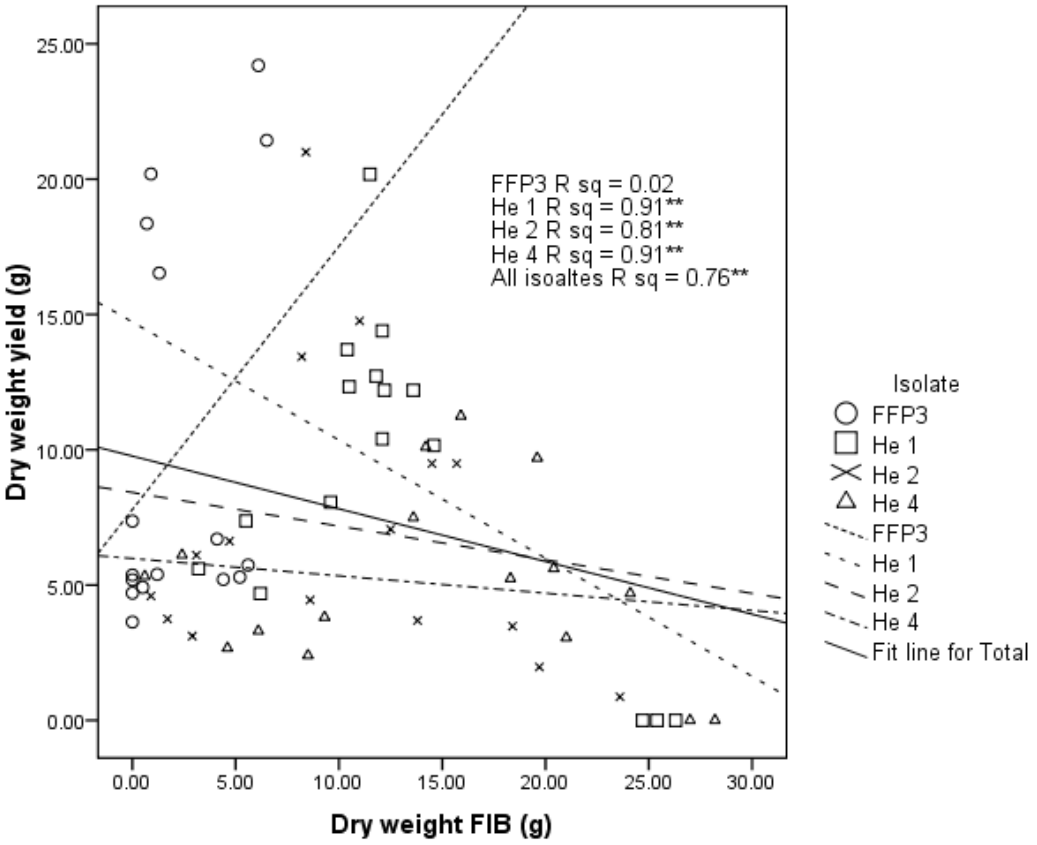

Figure 3.22 Regression of dry weight yield of mushrooms by dry weight of malformed mushrooms produced in the bag (FIB) produced by four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust for exp 2d (four *Hericium* isolates, one sawdust type, using rye grain spawn).

Table 3.22 ANOVA standard least squares using REML[1] method for total fresh and dry weight of mushrooms produced by four isolates of *Hericium* on supplemented *Acer rubrum* sawdust for exp 2d (using rye grain spawn)

Response	Source	DF	F-Ratio	Prob > F
Total fresh weight				
	Isolate	3	5.4216	0.0023*
Total dry weight				
	Isolate	3	0.2155	0.8852

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level. [1] restricted maximal likelihood

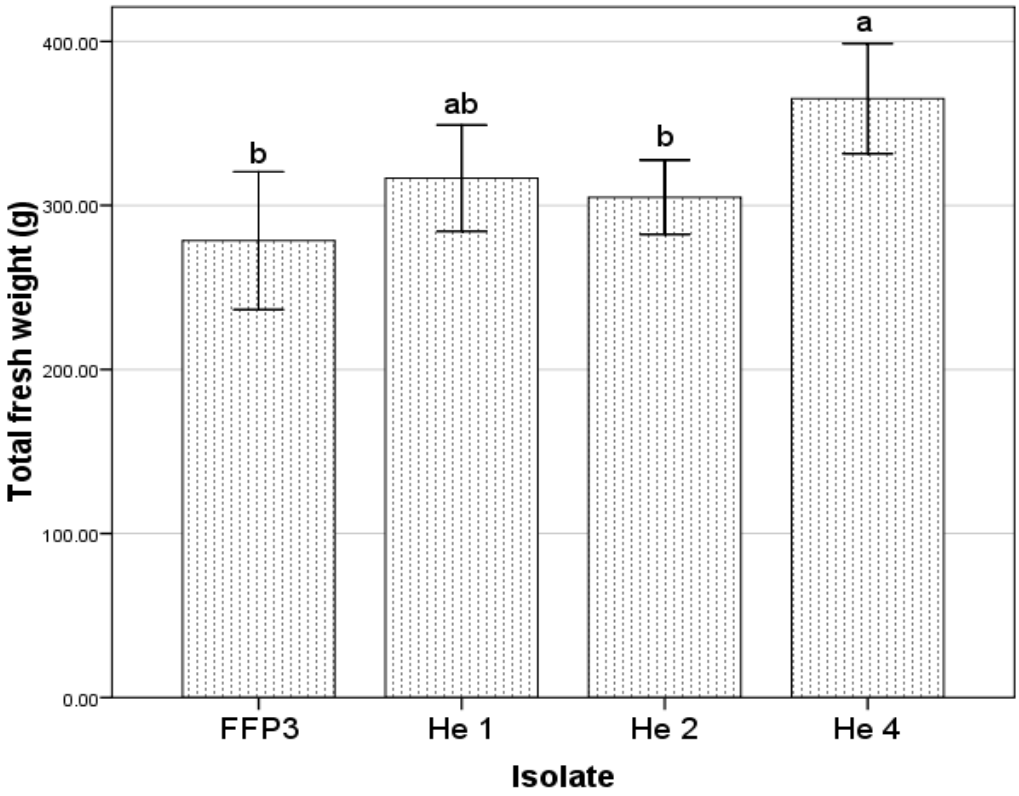

Figure 3.23 Comparison of total fresh weight (fresh weight yield combined with fresh weight of mushrooms produced in the bag (FIB)) for four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected H*. americanum* isolates) grown on wheat bran supplemented sawdust from *Acer rubrum* exp 2d (four *Hericium* isolates, one sawdust substrate, rye grain spawn). Levels not connected by the same letter are significantly different (p < 0.05). Error bars: +/- 2 SE

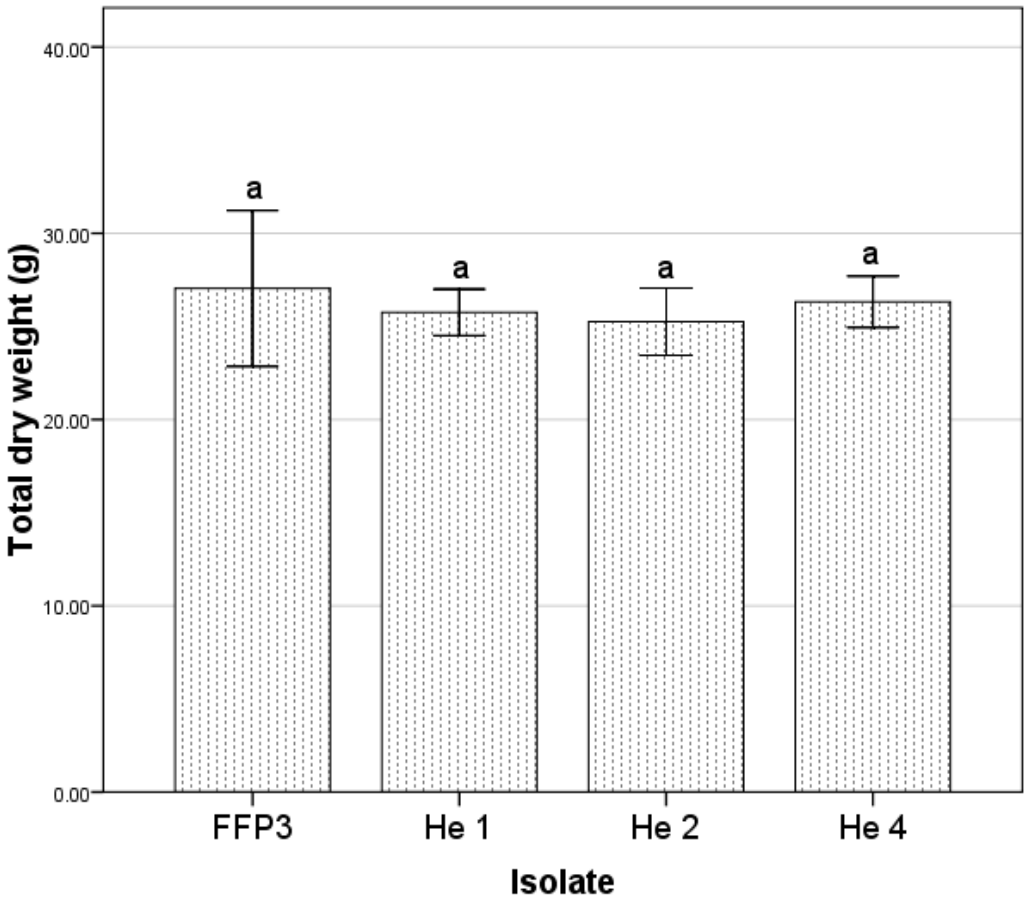

Figure 3.24 Comparison of total dry weight (dry weight yield combined with dry weight of mushrooms produced in the bag (FIB)) for four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He 2 & He 4 wild collected *H. americanum* isolates) grown on wheat bran supplemented sawdust from *Acer rubrum* exp 2d (four *Hericium* isolates, one sawdust substrate, rye grain spawn). Levels not connected by the same letter are significantly different ($p < 0.05$). Error bars: +/- 2 SE

3.4.2.6 Postharvest: Weight loss

For this portion of the experiment the data were analyzed by day (day 3, 6, 9) and data from the first two experiments, exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn) and 2c (four *Hericium* isolates, two sawdust types, millet grain spawn) were combined. The data from exp 2d (four *Hericium* isolates, one sawdust type, rye grain spawn) could not be included at this point because only one kind of sawdust was used. The data required a log transformation. Block and experiment were included as random effects. Block had a very small effect and experiment had no effect at all. Only the interaction of isolate x substrate was significant ($p < 0.05$) for all days (Table 3.23). Figure 3.25 shows the interaction between isolate and substrate by day. It seems that for one isolate percent weight loss is greater when the mushrooms were produced on Fg (FFP3) sawdust, one isolate shows greater weight loss when grown on Ar (He 1) and other isolates seem to show no difference between substrate (He 2 & He 4).

To further explore the data separate models were run by substrate. When the data are separated by substrate, data from exp 2d (four *Hericium* isolates, one sawdust type, rye grain spawn) could be combined with the data from other experiments. Isolate was significant for percent weight loss of mushrooms when grown on Ar sawdust for all days (Table 3.24). The separation of means can be seen in Figure 3.26. It appears that the hypothesis that mushrooms from *H. americanum* has higher percent weight loss cannot be rejected. The wild isolate He 4 consistently had higher weight loss compared to the commercial isolate of *H. erinaceus* (FFP3). In addition, mushrooms from He 1 had significantly higher weight loss compared to the commercial isolate, FFP3, on days 6 and 9.

Table 3.23 ANOVA standard least squares using REML[1] method for percent weight loss over 3, 6 or 9 days of mushrooms from 4 *Hericium* isolates grown on two different substrates (exp 2b & 2c only).

Day	Source	DF	F-Ratio	Prob > F
3				
	Isolate	3	1.5203	0.2096
	Substrate	1	0.2689	0.6045
	Isolate x Substrate	3	3.0736	0.0283*
6				
	Isolate	3	2.1245	0.0975
	Substrate	1	0.0286	0.8658
	Isolate x Substrate	3	3.9013	0.0094*
9				
	Isolate	3	1.9228	0.1263
	Substrate	1	0.0704	0.7909
	Isolate x Substrate	3	3.1001	0.0273*

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level. [1] restricted maximal likelihood

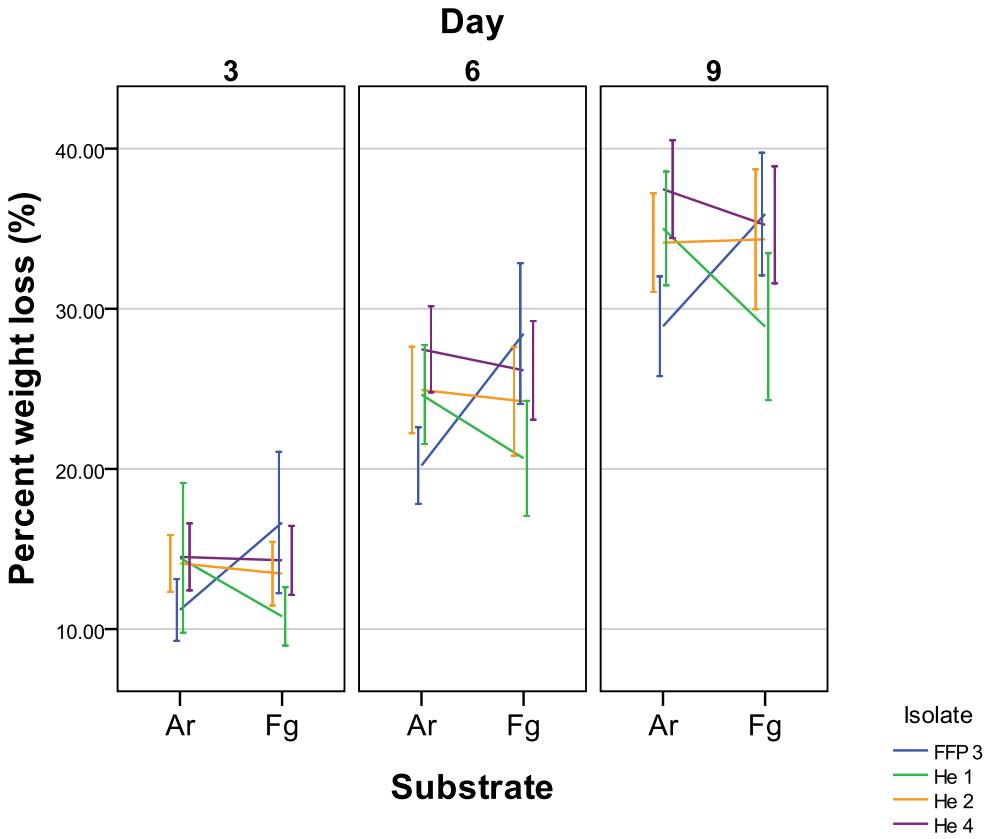

Figure 3.25 Comparison of postharvest percent weight loss of *Hericium* mushrooms from four isolates (FFP3 a commercial isolate of *H. erinaceus*, He1, He 2, He 4 wild isolates of *H. americanum*) grown on two sawdust substrates, *Acer rubrum* (Ar) and *Fagus grandifolia* (Fg). Error bars represent +/- 2 SE.

Table 3.24 ANOVA standard least squares using REML[1] method for percent weight loss over 3, 6 or 9 days of mushrooms from 4 *Hericium* isolates grown on two different substrates, *Acer rubrum* (Ar) or *Fagus grandifolia* (Fg) sawdust.

Substrate	Day	Source	DF	F-Ratio	Prob > F
Ar					
	3				
		Isolate	3	3.9275	0.0090*
	6				
		Isolate	3	6.8409	0.0002**
	9				
		Isolate	3	6.4996	0.0003**
Fg					
	3				
		Isolate	3	2.1514	0.0968
	6				
		Isolate	3	1.9922	0.1182
	9				
		Isolate	3	1.8844	0.1353

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level. [1] restricted maximal likelihood

Isolate was not significant for Fg for any day, although, it does appear that FFP3 had a higher percent weight loss especially for day 3 and 6 (Table 3.24). The difference may have become detectable if there were more replications. Fg only had 140 observations while Ar had 286; this is because exp 2d used only Ar sawdust. After examining Figure 3.27 it is suggested that with increased replication and reduced variability statistical differences may be seen among some of the isolates.

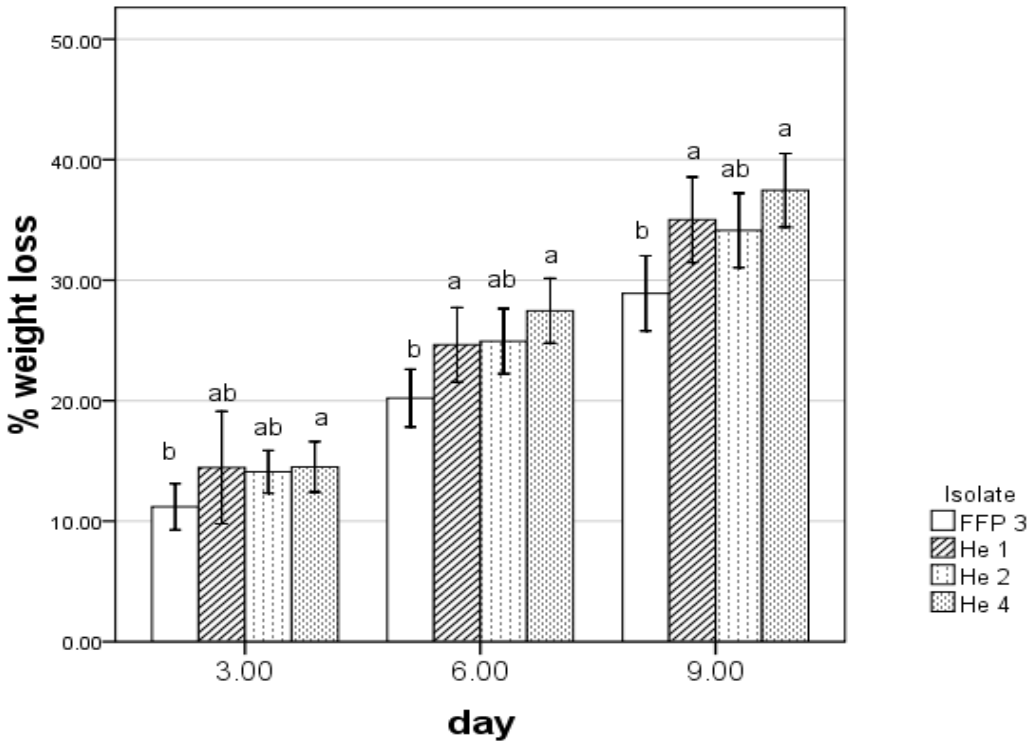

Figure 3.26 Percent weight loss by day of mushrooms from four *Hericium* (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on supplemented *Acer rubrum* sawdust for combined data from exp 2b, 2c & 2d. Error bars: +/- 2 SE

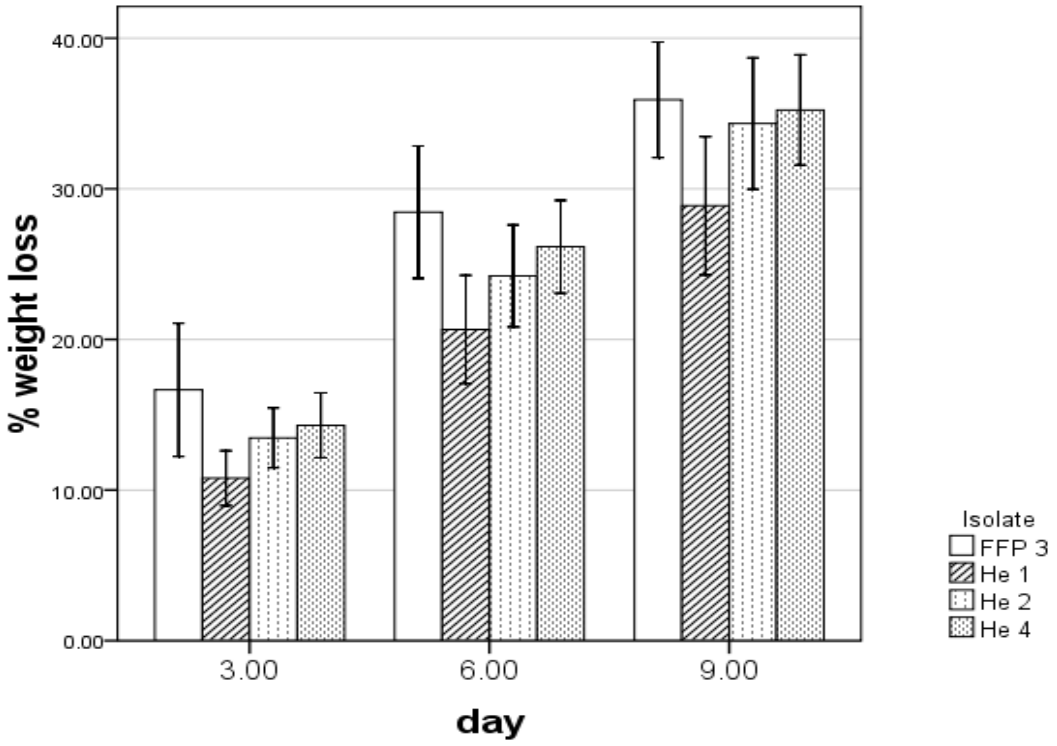

Figure 3.27 Percent weight loss by day of mushrooms from four *Hericium* (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) grown on supplemented *Fagus grandifolia* sawdust for combined data from exp 2b & 2c. Error bars: +/- 2 SE

3.4.2.7 Postharvest: Quality

The data from the three experiments (2b, 2c & 2d) were analyzed separately. The subjective quality rating scale used for experiment 2b had a 1- 4 rating. For this experiment the rating criteria for 4 and 5 were combined to be simply rated as 4. The distinction between the number 4 and 5 ratings were created after exp 2b was completed and was a rating scale of 1 – 5 was used for the subsequent experiments (exp 2c & 2d).

Experiment 2b (four Hericium isolates, two sawdust types, rye grain spawn)

The ordinal logistics model shows isolate and block to be significant on all days while the interaction of isolate x substrate was significant on all days except day 9 (Table 3.25). A generalized linear model was used to find significant differences in the quality ratings between the isolates. Separate models were run by day and substrate (Table 3.26). The separation of means can be seen in Figures 3.28 & 3.29. For mushrooms grown on Fg sawdust no significant difference among the isolates could be detected in the quality ratings except for day 0. On day zero FFP3, the commercial isolate, and He 4 were significantly better compared to only He 1.

There are more significant differences between the quality ratings of mushrooms grown on Ar sawdust. On day zero FFP3 has significantly higher quality ratings compared to only He 2, but on day 3 FFP3 has significantly higher quality rating compared to both He 2 and He 4. By day 6 FFP3 mushrooms had a significantly higher quality rating compared to all the wild collected isolates. This significance continues through day 9.

Table 3.25 Ordinal logistics model for mushroom quality of four isolates of *Hericium* grown on *Acer rubrum* (Ar) or *Fagus grandifolia* (Fg) sawdust using rye grain spawn (experiment 2b)

Day	Source	DF	Chi-square	Prob > Chi-square
0				
	Isolate	3	14.485844	0.0023*
	Substrate	1	0.27011198	0.6033
	Isolate x substrate	3	12.5519415	0.0057*
	Block	7	28.4654243	0.0002**
3				
	Isolate	3	18.9264862	0.0003**
	Substrate	1	2.1678464	0.1409
	Isolate x substrate	3	18.2685668	0.0004**
	Block	7	19.2760219	0.0074*
6				
	Isolate	3	29.5126587	<.0001**
	Substrate	1	0.71968616	0.3962
	Isolate x substrate	3	14.8124267	0.002*
	Block	7	14.8507396	0.038*
9				
	Isolate	3	31.5192077	<.0001**
	Substrate	1	0.00767168	0.9302
	Isolate x substrate	3	7.7709466	0.051
	Block	7	18.3845683	0.0104*

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level.

Table 3.26 Results of contrast to compare the quality ratings of mushrooms from four *Hericium* isolates over time grown on *Acer rubrum* (Ar) or *Fagus grandifolia* (Fg) sawdust for exp 2b (four Hericium isolates, two sawdust types, rye grain spawn). Adjusted Prob> Chi-square corrects for Bernoulli multiple comparisons by multiplying Prob > Chi-square by the number of comparisons (6).

Day	Substrate	Isolate	Chi-square	Prob > Chi-square	Adjusted Prob > Chi-square
0	Ar				
		FFP3 > He 1	2.4	0.1211	0.7266
		FFP3 > He 2	11.3	0.0008	0.0048*
		FFP3 > He 4	6.19	0.0129	0.0774
		He 1 > He 2	1.38	0.2396	1.4376
		He 1 > He 4	0.11	0.7438	4.4628
		He 2 < He 4	2.13	0.1448	0.8688
3	Ar				
		FFP3 >. He 1	5.14	0.0234	0.1404
		FFP3 > He 2	16.09	<0.0001	<0.0001**
		FFP3 > He 4	9.82	0.0017	0.0102*
		He 1 > He 2	1.81	0.1788	0.1073
		He 1 > He 4	0.08	0.7831	4.6986
		He 2 < He 4	2.89	0.0892	0.5352
6	Ar				
		FFP3 > He 1	14.07	0.0002	0.0012*
		FFP3 > He 2	21.8	<0.0001	<0.0001**
		FFP3 > He 4	16.01	<0.0001	<0.0001**
		He 1 > He 2	0.06	0.8103	4.8618
		He 1 < He 4	0.76	0.3848	2.309
		He 2 < He 4	2.23	0.1352	0.8112
9	Ar				
		FFP3 > He 1	13.49	0.0002	0.0012*
		FFP3 > He 2	19.84	<0.0001	<0.0001**
		FFP3 > He 4	17.36	<0.0001	<0.0001**
		He 1 < He 2	0.4	0.5262	3.1572
		He 1 < He 4	1.48	0.2236	1.3416
		He 2 < He 4	0.71	0.4	2.4
0	Fg				
		FFP3 > He 1	6.96	0.0003	0.0018*
		FFP3 < He 2	0.1	0.7501	4.5006
		FFP3 < He 4	0.97	0.3243	1.9458
		He 1 < He 2	4.64	0.0313	0.1878
		He 1 < He 4	8.09	0.0045	0.027*
		He 2 < He 4	1.26	0.2616	1.5696
3	Fg				
		FFP3 > He 1	4.1	0.0428	0.2568
		FFP3 < He 2	0.01	0.915	5.49
		FFP3 < He 4	0.58	0.4473	2.6838
		He 1 > He 2	3.79	0.0514	0.3084
		He 1 > He 4	5.33	0.021	0.126
		He 2 < He 4	0.36	0.5475	3.285
6	Fg				
		FFP3 > He 1	4.73	0.0296	0.1776
		FFP3 < He 2	0.1	0.7488	4.4928
		FFP3 < He 4	0.12	0.7297	4.3782
		He 1 > He 2	3.24	0.0721	0.4326
		He 1 > He 4	2.93	0.087	0.522
		He 2 < He 4	0	0.9717	5.8302
9	Fg				
		FFP3 > He 1	5.71	0.0169	0.1014
		FFP3 < He 2	1.23	0.2668	1.6008
		FFP3 < He 4	1.14	0.2857	1.7142
		He 1 > He 2	1.93	0.1653	0.9918
		He 1 > He 4	1.91	16.75	100.5
		He 2 > He 4	0	0.9798	5.8788

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level.</> indicates average quality rating for a given isolate is < or > than the average quality rating of the isolate to which it was compared.

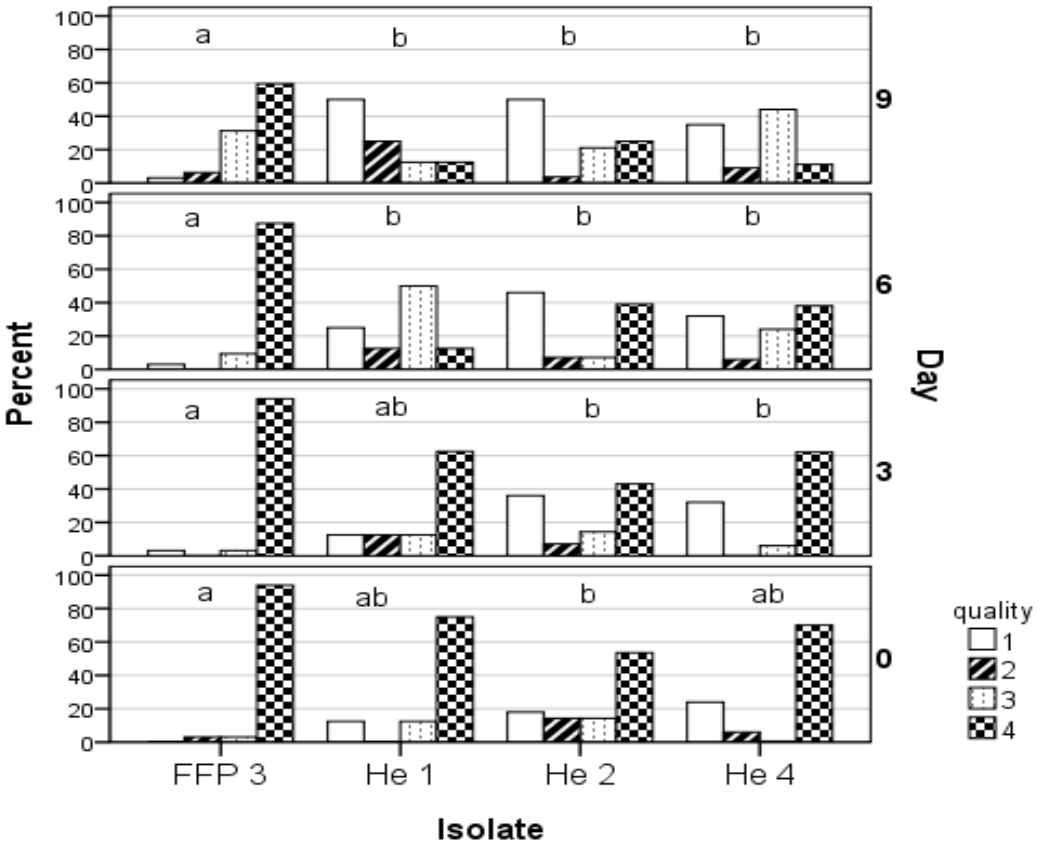

Figure 3.28 Comparison of mushroom quality over 9 days of 4 *Hericium* isolates grown on *Acer rubrum* (Ar) sawdust with rye grain spawn for experiment 2b (four Hericium isolates, two sawdust types, rye grain spawn) using a descending subjective quality rating scale (1= lowest quality, 4= best quality). Isolates not connected by the same letter *within day* are significantly different ($p<0.05$).

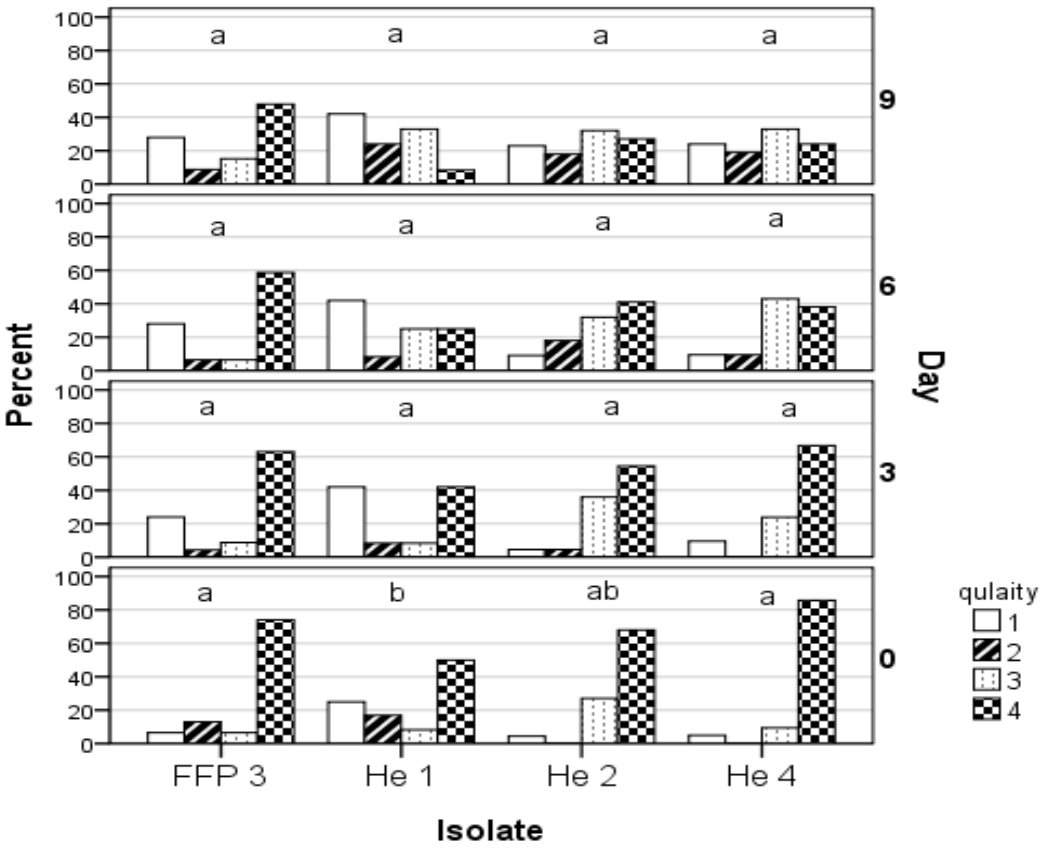

Figure 3.29 Comparison of mushroom quality over 9 days of 4 *Hericium* isolates grown on *Fagus grandifolia* (Fg) sawdust with rye grain spawn (experiment 2b) using a descending subjective quality rating scale(1= lowest quality, 4= best quality). Isolates not connected by the same letter within day are significantly different ($p<0.05$).

Experiment 2c (four Hericium isolates, two sawdust types, millet grain spawn)

The ordinal logistics model shows isolate and block to be significant for each day and the interaction of isolate x substrate to be significant on day 6 and 9 (Table 3.27). A generalized linear model was used to find significant differences in the quality ratings between the isolates. Separate models were run by day and substrate. The significant differences between isolates can be seen in Table 3.28 and Figures 3.30 and 3.31. For mushrooms produced from on Ar sawdust on day 0 and day 3, He 1 mushrooms had a significantly lower quality rating from the other three isolates. There was no significant difference

in the quality rating by isolate for day 6 and day 9.

For mushrooms produced from Fg sawdust, He 1 and He 4 have significantly lower quality ratings compared to mushrooms produced by the commercial isolate, FFP3 on day 0 and day 3. On day 6 only mushrooms produced by He 4 are significantly lower quality rating compared to those of commercial isolate, FFP3. There is no significant difference between the quality ratings of the isolates on day 9.

Table 3.27 Ordinal logistics model for mushroom quality of four isolates of *Hericium* grown on *Acer rubrum* (Ar) or *Fagus grandifolia* (Fg) sawdust using millet grain spawn (experiment 2c)

Day	Source	DF	Chi-square	Prob > Chi-square
0				
	Isolate	3	42.1506945	<0.0001**
	Substrate	1	0.98330981	0.3214
	Isolate x substrate	3	4.40138652	0.2213
	Block	7	20.9276021	0.0039*
3				
	Isolate	3	39.295706	<0.0001**
	Substrate	1	0.42199176	0.5159
	Isolate x substrate	3	6.294479	0.0981
	Block	7	16.1698234	0.0236*
6				
	Isolate	3	38.6382405	<.0001**
	Substrate	1	3.52309859	0.065
	Isolate x substrate	3	11.3340854	0.0100*
	Block	7	20.8882975	0.0039*
9				
	Isolate	3	30.6924788	<.0001**
	Substrate	1	1.80107564	0.1796
	Isolate x substrate	3	8.83360144	0.0316*
	Block	7	17.8824734	0.0125*

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level. </> indicates average quality rating for a given isolate is < or > than the average quality rating of the isolate to which it was compared.

Table 3.28 Results of contrast to compare the quality ratings of mushrooms from four *Hericium* isolates over time grown on *Acer rubrum* (Ar) or *Fagus grandifolia* (Fg) sawdust using millet grain sawdust (experiment 2c). Adjusted Prob> Chi-square corrects for Bernoulli multiple comparisons by multiplying Prob > Chi-square by the number of comparisons (6).

Day	Substrate	Isolate	Chi-square	Prob > Chi-square	Adjusted Prob > Chi-square
0	Ar				
		FFP3 > He 1	18.92	<0.0001	<0.0001**
		FFP3 > He 2	3.11	0.0779	0.4674
		FFP3 > He 4	6.50	0.0108	0.0648
		He 1 < He 2	13.82	0.0002	0.0012*
		He 1 < He 4	10.03	0.0015	0.0090*
		He 2 > He 4	1.71	0.1915	1.1490
3	Ar				
		FFP3 > He 1	18.99	<0.0001	<0.0001**
		FFP3 > He 2	6.83	0.009	0.0540
		FFP3 > He 4	6.48	0.0109	0.0654
		He 1 < He 2	9.81	0.0017	0.0102*
		He 1 < He 4	8.88	0.0029	0.0174*
		He 2 < He 4	0.01	0.9164	5.4984
6	Ar				
		FFP3 > He 1	0.00	0.09999	0.5999
		FFP3 > He 2	5.44	0.0197	0.1182
		FFP3 > He 4	6.15	0.0131	0.0786
		He 1 < He 2	0.00	0.9999	0.5999
		He 1 < He 4	0.00	0.9999	0.5999
		He 2 < He 4	0.23	0.6326	3.7956
9	Ar				
		FFP3 > He 1	0	0.9999	0.5999
		FFP3 > He 2	4.19	0.0406	0.2436
		FFP3 > He 4	4.19	0.0408	0.2448
		He 1 < He 2	0.00	0.9999	0.5999
		He 1 < He 4	0.00	0.9999	0.5999
		He 2 < He 4	0.00	0.9752	5.8512
0	Fg				
		FFP3 > He 1	14.43	0.0001	<0.0001**
		FFP3 > He 2	6.49	0.0108	0.0648
		FFP3 > He 4	13.65	0.0002	0.0012*
		He 1 <. He 2	4.77	0.029	0.1740
		He 1 < He 4	1.6	0.2056	1.2336
		He 2 > He 4	2.47	0.1162	0.6972
3	Fg				
		FFP3 > He 1	8.67	0.0032	0.0192*
		FFP3 > He 2	1.97	0.1603	0.9618
		FFP3 > He 4	9.84	0.0017	0.0102*
		He 1 < He 2	2.17	0.141	0.8460
		He 1 < He 4	0.56	0.454	2.7240
		He 2 > He 4	1.17	0.2791	1.6746
6	Fg				
		FFP3 > He 1	6.05	0.0139	0.8340
		FFP3 > He 2	0.49	0.4831	2.8986
		FFP3 > He 4	9.09	0.0026	0.0156*
		He 1 < He 2	2.14	0.1439	0.8634
		He 1 < He 4	0.00	0.9958	5.9748
		He 2 > He 4	2.67	0.1023	0.6138
9	Fg				
		FFP3 > He 1	6.1	0.0135	0.0810
		FFP3 = He 2	0.00	0.9786	5.8716
		FFP3 > He 4	5.98	0.0145	0.0870
		He 1 < He 2	4.34	0.0371	0.2226
		He 1 < He 4	0.28	0.5940	3.5640
		He 2 > He 4	3.63	0.5680	3.4080

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level. </> indicates average quality rating for a given isolate is < or > than the average quality rating of the isolate to which it was compared.

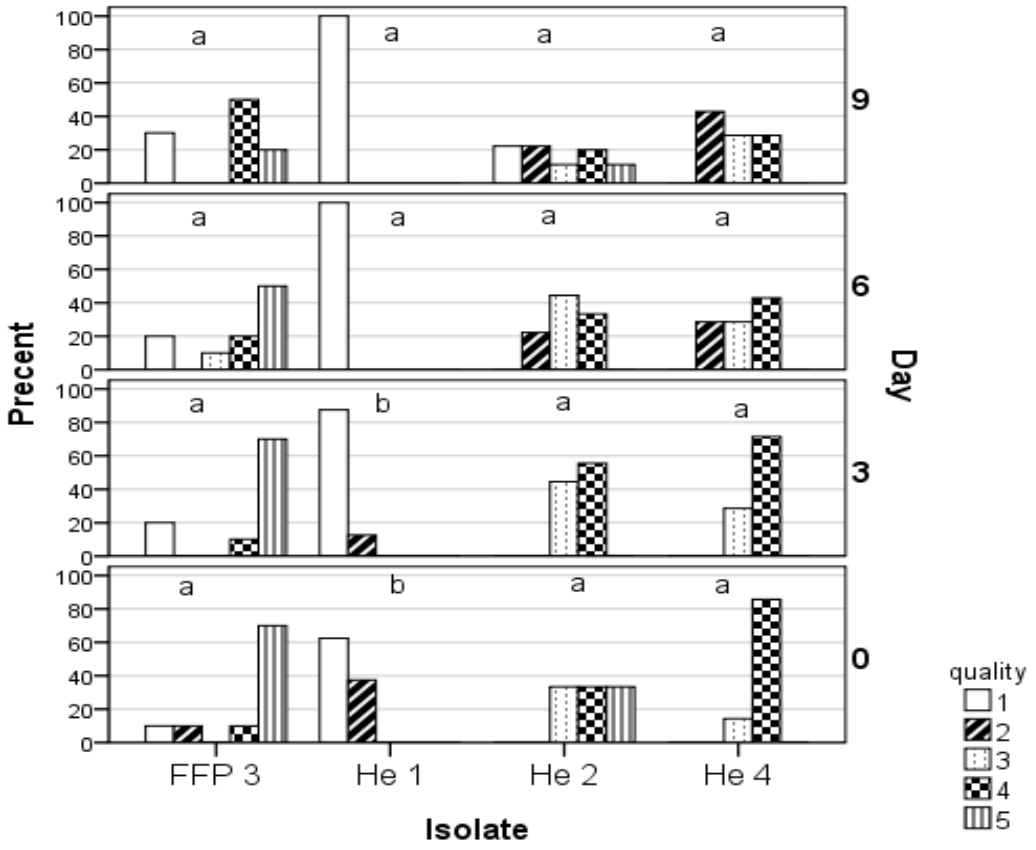

Figure 3.30 Comparison of mushroom quality over 9 days of 4 *Hericium* isolates grown on *Acer rubrum* (Ar) sawdust with millet grain spawn (experiment 2c) using a descending subjective quality rating scale (1= worst quality, 5= best quality). Isolates not connected by the same letter *within day* are significantly different ($p<0.05$).

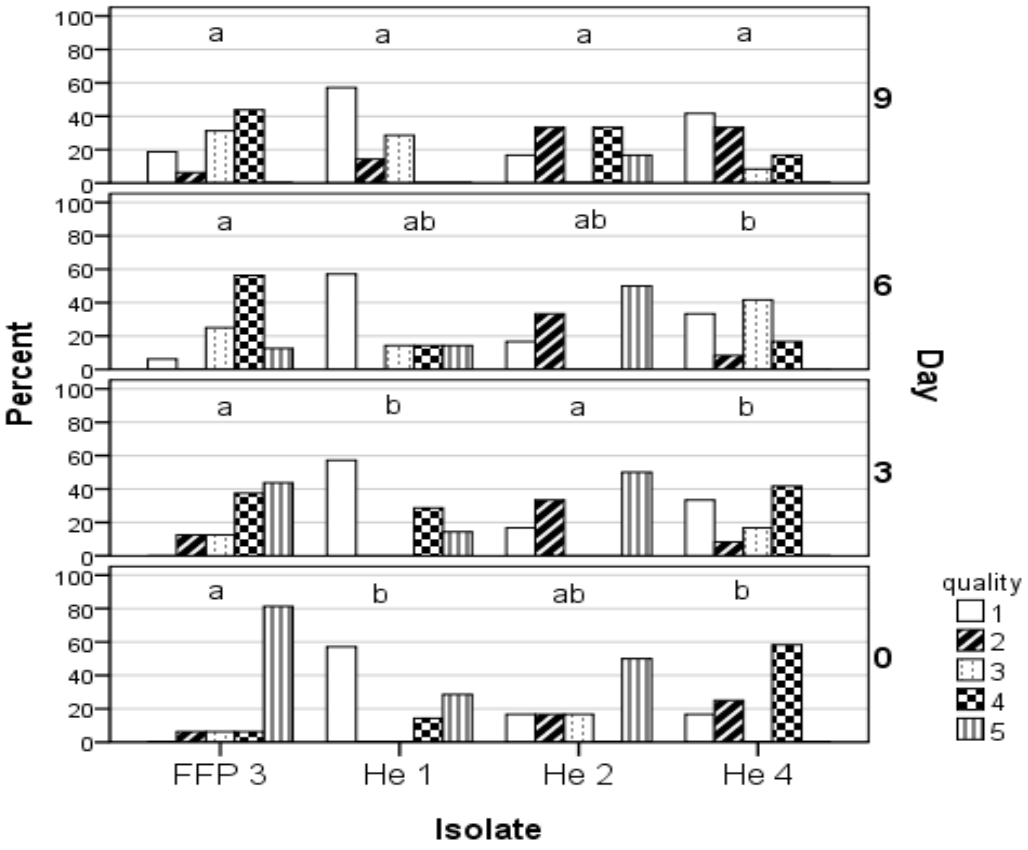

Figure 3.31 Comparison of mushroom quality over 9 days of 4 *Hericium* isolates grown on *Fagus grandifolia* (Fg) sawdust with millet grain spawn (experiment 2c) using a descending subjective quality rating scale (1= worst quality, 5= best quality). Isolates not connected by the same letter within day are significantly different ($p<0.05$).

Exp 2d (four Hericium isolates, one sawdust type, rye grain spawn)

The ordinal logistics model shows isolate to be significant on each day except for day 3. Block was found to be significant for each day (Table 3.29). A generalized linear model was used to find significant differences in the quality ratings of the isolates. Separate models were run by day. The separation of means can be seen in Table 3.30 and Figure 3.32. On day 0 and mushrooms produced by isolate He 1 have significantly lower quality ratings compared to mushrooms produced by all the other isolates. There was no significant difference between the mushrooms produced by any of the isolates on day 3. On day 6 mushrooms produced by He 1 have a significantly lower quality rating than the commercial isolate, FFP3. By day 9 mushrooms produced by He 1 had a significantly lower quality compared to mushrooms produced by FFP3 and He 2 while mushrooms produce by He 2 have a significantly better quality rating compared to mushrooms produced from He 1.

Table 3.29 Ordinal logistics model for mushroom quality of four isolates of *Hericium* grown on *Acer rubrum* (Ar) sawdust for exp 2d (four *Hericium* isolates, one sawdust type, rye grain spawn)

Day	Source	DF	Chi-square	Prob > Chi-square
0				
	Isolate	3	12.2875144	0.0065**
	Block	7	33.4175106	<0.0001**
3				
	Isolate	3	7.75151553	0.0514
	Block	7	14.0519302	0.0503**
6				
	Isolate	3	17.2927821	0.0006*
	Block	7	21.5479533	0.0030**
9				
	Isolate	3	28.1647548	<.0001**
	Block	7	17.89067	0.0125**

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level.

Table 3.30 Results of contrast to compare the quality ratings of mushrooms from four *Hericium* isolates over time grown on *Acer rubrum* (Ar) sawdust experiment 2d (four *Hericium* isolates, one sawdust type, rye grain spawn). Adjusted Prob> Chi-square corrects for Bernoulli multiple comparisons by multiplying Prob > Chi-square by the number of comparisons (6).

Day	Substrate	Isolate	Chi-square	Prob > Chi-square	Adjusted Prob > Chi-square
0	Ar				
		FFP3 > He 1	7.02	0.0081	0.0486*
		FFP3 < He 2	0.01	0.9071	5.4426
		FFP3 < He 4	0.87	0.3504	2.1024
		He 1 < He 2	7.58	0.0059	0.0354*
		He 1 < He 4	8.87	0.0029	0.0174*
		He 2 < He 4	0.69	0.4072	2.4432
3	Ar				
		FFP3 > He 1	6.18	0.0129	0.0774
		FFP3 > He 2	2.73	0.0985	0.5910
		FFP3 > He 4	4.13	0.0420	0.2520
		He 1 < He 2	1.23	0.2682	1.6092
		He 1 < He 4	0.7	0.4042	2.4252
		He 2 < He 4	0.11	0.7376	4.4256
6	Ar				
		FFP3 > He 1	11.68	0.0006	0.0036*
		FFP3 > He 2	1.82	0.1775	1.0650
		FFP3 > He 4	11.28	0.0008	0.0048
		He 1 < He 2	4.69	0.0304	0.1824
		He 1 < He 4	0.48	0.4873	2.9238
		He 2 > He 4	3.08	0.0791	0.4746
9	Ar				
		FFP3 > He 1	18.69	<0.0001	<0.0001**
		FFP3 > He 2	2.64	0.1042	0.6252
		FFP3 > He 4	18.51	<0.0001	<0.0001**
		He 1 < He 2	8.14	0.0043	0.0258*
		He 1 < He 4	0.85	0.3578	2.1468
		He 2 > He 4	5.65	0.0175	0.1050

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level. </> indicates average quality rating for a given isolate is < or > than the average quality rating of the isolate to which it was compared.

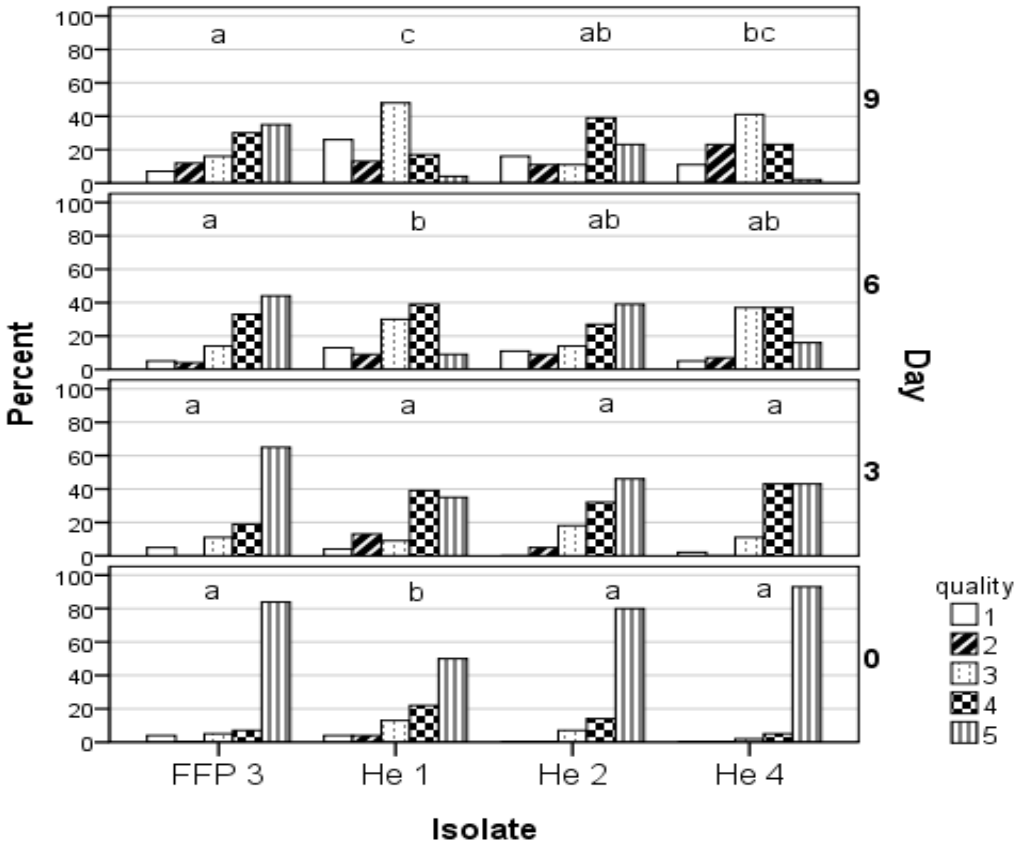

Figure 3.32 Comparison of mushroom quality over 9 days of 4 *Hericium* isolates grown on *Acer rubrum* (Ar) sawdust with rye grain spawn (experiment 2d) using a descending subjective quality rating scale (1= worst quality, 5= best quality). Isolates not connected by the same letter *within day* are significantly different ($p<0.05$).

3.4.3 Exp 3: Totem results

Data was collected from April 2008 through the end of July 2009. Out of the 128 logs involved in the experiment, only 39 produced mushrooms. A nominal logistics model found isolate to be a significant predictor in the presence of mushrooms (Table 3.31). A Chi- square test shows that He 3 had significantly more logs that produced mushrooms (Table 3.32). Figure 3.33 shows the percentage of logs that produced at least one mushroom for each isolate.

Because only approximately 30% of the logs produced mushrooms, the yields were low and the means could not be statistically analyzed.

Table 3.31 Results of nominal logistics model of presence/absence of *Hericium* mushrooms for exp 3.

Source	DF	Chi-square	p-value
Isolate	3	19.850	0.0002*
Block	3	1.2001	0.7530

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level.

Table 3.32 Chi-Square test of presence/absence of mushrooms for four Hericium isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He 4 wild collected *H. americanum* isolates) for exp 3.

Isolate	Estimate	Chi-square	p-value
FFP3	0.5489	2.02	0.1555
He 3	-1.4517	18.43	<0.0001**
He 4	0.354	0.91	0.341
He 5	0.549	2.02	0.1555

*denotes significance at p = 0.05 level. **denotes significance at p = 0.001 level.

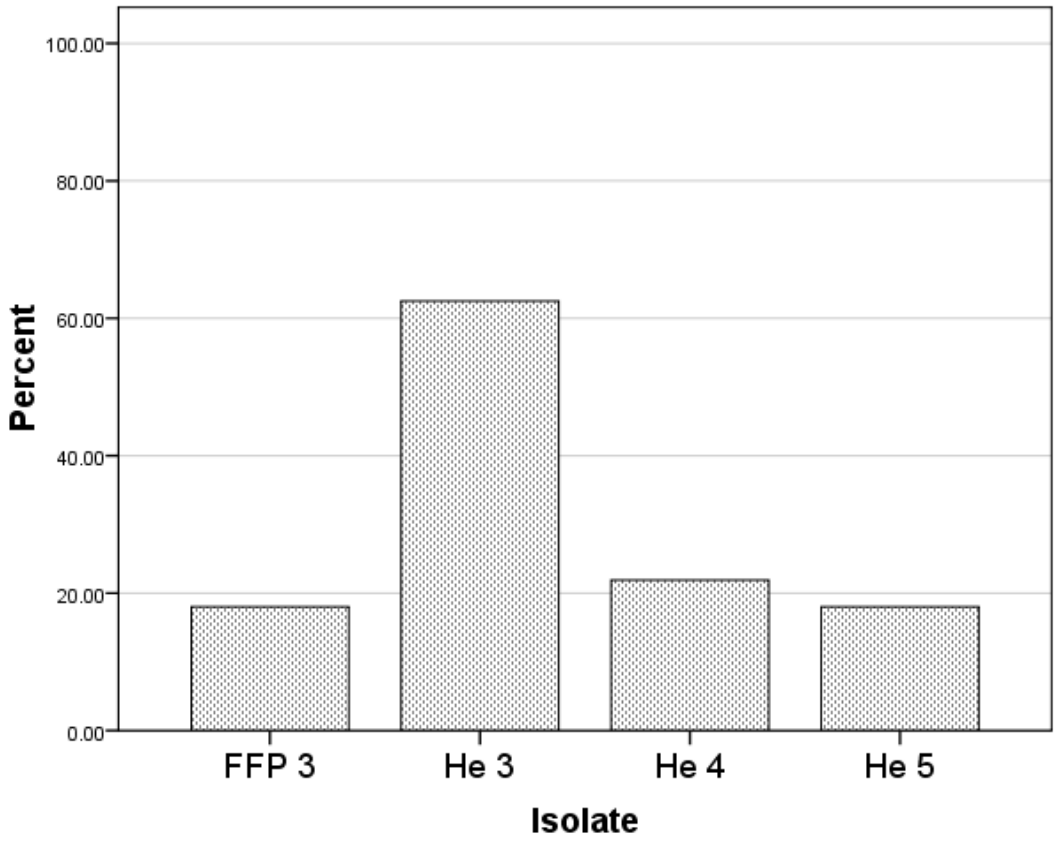

Figure 3.33 Percentage of logs that produced at least one mushroom for four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) for exp 3

3.5 Discussion

3.5.1 Mycelial growth on PDA

A wide range in colony diameters was found among the isolates. That there are differences in optimal growth temperature among isolates of the same species reflects the conflicting reports of optimal temperature reported in the literature (Ko et al 2005, Ginns 1985, Imtiaj 2008). Ginns (1985) tested the growth of 11 *H. americanum* isolates and 12 isolates of *H. erinaceus.* By using such a large number of isolates the researchers were able to determine a general optimal temperature for each species but also noted that there was a wide range in growth rates between the isolates within species. While our

investigation used a much more modest number of isolates, the observation made by Ginns (1985) reflects the range in growth rates among isolates that we found.

We found the fastest growth for both *H. americanum* and *H. erinaceus* was generally found to be at 25^0C. Some isolates (FFP3, He 2, He 6) experienced reduced growth at the highest temperature (30^0C) while other isolates (He 1, He 3, He 4 & He 5) were not affected by the temperature increase. He 1 actually displayed an increase in growth (although not significant) at 30^0C as compared to 25^0C. It would be interesting to see what temperature He 1 could tolerate before a negative effect on growth was observed. This is similar to findings by Imtiaji (2008), who tested 4 isolates of *H. erinaceus* and found that while all the isolates had the fastest growth at 25^0C one isolate showed no difference in growth between 20^0C and 25^0C. However, most isolates showed a reduced growth with the small 5^0C increase from 25^0C to 30^0C. This agrees with our findings and shows the importance of testing new cultures at a wide range of temperatures.

This reduced sensitivity to temperature increases found in some *Hericium* isolates suggests some isolates have a wider range in optimal temperature. This may be a desirable characteristic for isolates used in commercial mushroom production as well as outdoor production where there is little growers can do to control temperature fluctuations. When evaluating wild isolates for outdoor cultivation it seems that finding an isolate that displays a wide optimal temperature range may be more important than finding an isolate that has extremely fast growth, especially if that fast growth is only at a specific temperature. This is yet another reason for researchers to evaluate the growth rate of new isolates across a range of temperatures.

As we expected the *Hericium erinaceus* isolate, FFP3, showed the slowest growth, compared to most of the wild isolates of *H. americanum* as found by Ko et al. (2005) and Ginns (1985), who both found the growth of *H. americanum* to be faster than that of *H. erinaceus*. *H. erinaceus* did not display the fastest growth at any of the temperatures, however, wild isolate He 2 showed similarly slow growth. Therefore, it cannot be assumed that all *H. americanum* cultures will grow faster than *H. erinacues* cultures.

The faster growth of the majority of wild isolates of *H. americanum* compared to that of an isolate of *H. erina*ceus that is currently being used in commercial production suggests that an isolate of *H. americanum* could one day be used in commercial production of mushrooms. It has been suggested by Stamets (2000) and Zervakis (2001) that the rate of mycelial growth and subsequent speed of substrate colonization has a direct impact on the success of an isolate in a commercial production setting. It is suggested that isolates with faster growth rate colonize substrate faster, reducing the chance for uncolonized substrate becoming contaminated. Faster colonization of the substrate should also reduce the time to fruiting. If our hypothesis that fast mycelial growth is also correlated with mushroom production had been correct, one of these wild collected isolates could, in fact, be a viable isolate for commercial production of *Hericium* mushroom.

3.5.2 Fresh and dry weight yield

Fast mycelial growth rate has been associated with fast colonization of substrate, reduced substrate contamination (Zervakis 2001) and shorter time to fruiting (Stamets 2000). We wrongly suspected that fast *in vitro* growth rate on PDA would also associate with high mushroom yield.

Overall, we did not find a link between fast *in vitro* growth and high yield based on both fresh and dry weight yields. While fast growth does seem like an important characteristic for a commercially viable isolate, it is evidently not the most important factor. He 1, the isolate with the fastest *in vitro* growth, was routinely shown to have among the lowest mushroom yields. In fact, the commercial variety, which showed relatively slow growth *in vitro*, tended to have the highest dry and fresh weight yields. This could lead us to hypothesize that fast growth rate is actually inversely related to yield. However He 2, the wild isolate with the slowest *in vitro* growth, was another wild isolate that had fairly low yields. The association between *in vitro* growth and mushroom yield should be further explored in future experiments. Growth rate may have a different relationship with yield in the two *Hericium* species.

In a study of *Pleurotus ostreatus* the researchers suggested that “when a culture medium with a simple carbohydrate is used, the [fungus] becomes less efficient and metabolically non viable”, which they concluded would result in lower yield. Therefore, growth on a substrate with a simple carbohydrate, like PDA, may not be indicative of growth on a substrate with a more complex food source (Rosado et al 2002). In addition to determining growth rates on PDA, it is common for investigators to use a linear growth test experiment using the substrate to be used in mushroom production to evaluate isolates (Curvetto et al 2002, Ko et al 2005, Lee et al 2004, Figlas et al 2007, Uhart 2008, Duncan 1991). This involves sterilizing a specific volume of the substrate to be tested in a glass tube and adding spawn or a piece of colonized agar to the tube. After incubation in the dark for several days, the distance of mycelial growth in the substrate (from the top of the tube to the actively growing edge) is measured at several equidistant points around the

tube. The measurements are then averaged. This method can be used to provide an estimate of the growth rate of strains as well as an estimate of a substrate's potential to support mycelial growth for a particular isolate. Perhaps growth rate determined by a linear growth test using the substrate that the fungus will be grown on during mushroom production would, in fact, be a better predictor of yield. However in a study by Zervakis (2001) the linear growth rates of 7 species of mushrooms were compared with yield produced on the substrates that showed the fastest mycelial growth. Only 3 out of the 7 isolates had the highest yield on the substrate that showed the fastest mycelial growth. The researchers went on to speculate that fast mycelial growth can often be "interoperated as an indication of hyphal progression on a nutritionally poor or unfavorable medium" as the mycelium searches for higher quality substrate. They go on to say that a slower denser growth of mycelium may suggest the fungus can better exploit the nutrient resource it is growing on.

When evaluating wild isolates for potential commercial use, it appears that the commercial variety tended to have the highest dry and fresh weight yields with the wild isolate He 4 appearing to perform equally as well in only one case (exp 2c the only experiment in which millet spawn was used). These results agree with those of Xiao and Chapman (1997) who found relatively low mushrooms yields from a wild collected isolate of *H. abietis* when cultivated on softwood sawdust.

Better environmental controls and an increased number of replications would have improved the statistical accuracy of our experiments and significant differences in means could probably be detected. Overall, the data were fairly variable and this variability may be inherent in this type of experiment. Alternatively, it could be that the variable environmental condition

of the growing room had an effect on the formation of mushrooms. As was previously mentioned the temperature and relative humidity of the growing room were not reliably controlled. Our observations suggest that mushroom formation is affected by the drastic relative humidity changes that occurred in this experiment. Drastic drops in relative humidity appeared to cause young mushrooms/primordia just beginning to emerge to halt development. Xiao and Chapman (1997) also noted a similar observation that as relative humidity dropped to 80% (from 90%) the mushrooms of *H. abietis* began to dry, brown and growth was poor or stopped all together. Since the isolates did not all develop at the same rate the random fluctuations in humidity could affect each isolate differently. For example, if a humidity drop occurs just as primordia from a particular isolate are forming outside of the bag, this may have a stronger effect than that seen in another isolate that has mushrooms further ahead or behind in development. In addition, even though bags of the same isolate tended to develop at similar rates they are not all exactly the same so the effect of a random relative humidity fluctuation is depends on the stage of development of the mushrooms on any one particular bag. This would increase bag to bag variability. If these drastic fluctuations could be avoided variability among bags may be reduced.

For most *Hericium* mushroom growing experiments and commercial growing operations two separate rooms with slightly different growing conditions are used (Ko 2005, Xiao and Chapman 1997, Figlas 2007, Stamets 2000). One room is typically used to encourage colonization of the substrate by the mycelium while suppressing primordia formation (spawn run). Once the substrate is satisfactorily colonized the bags are moved to the second room (fruiting room) which has conditions that induces primordia formation.

Alterations in temperature and atmospheric CO_2 levels between the two production rooms are utilized to suppress or encourage primordia growth at different stages in the cultivation process. We lacked the facilities for separating spawn run and fruiting.

3.5.3 Fruiting in the bag (FIB)

The FIB phenomenon, where unsalable fruiting bodies begin to form inside the bag, is clearly an issue for the wild collected isolates. In exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn) and 2d (four *Hericium* isolates, one sawdust types, rye grain spawn), in which rye was used for spawn, the wild isolates had greater FIB compared to the commercial isolate. There was no significant difference of the dry or fresh weight FIB among the isolates in exp 2c (four Hericium isolates, two sawdust types, millet grain spawn). Experiment 2c differed from the other two experiments in that millet grain was used for spawn. The change from rye grain spawn to millet grain spawn was a decision made after the observation that in exp 2b (four *Hericium* isolates, two sawdust types, rye grain spawn) an excessive amount of primordia had formed inside the bag rather than at the site of the holes that were poked in the bag. Dr. Daniel Royse, a researcher at Penn State University, suggested that primordia formation and the resulting FIB is a common result when grain spawn is not mixed thoroughly, resulting in uneven spawn distribution (pers. comm.). We also observed that primordia commonly formed at the site of a piece of rye grain spawn. By changing to smaller millet grain as the substrate for spawn we were attempting to allow for better spawn distribution. We also suspected that the smaller grain would be less likely to “support” the growth of primordia. By changing to millet grain spawn it was

hoped that FIB would be reduced. The distribution of spawn appeared to be improved with the millet grain spawn; however the occurrence of FIB did not appear to be reduced.

While the differences in mushroom yields and FIB between the experiments cannot be solely attributed to the change in grain spawn they are quite striking. The fresh weight yield of all the isolates was lower using millet spawn compared to the other two experiments in which rye spawn was used. While the weight of FIB for *wild* isolates does not seem to change that much between experiments, the FIB of the commercial isolate (FFP3) was affected by the use of millet grain. The fresh weight FIB for the commercial isolate, FFP3, is below 50 g in exp 2b and 2d (which used rye grain spawn), and was over 200 g when millet grain spawn was used in exp 2c. Also the yield of FFP3 in exp 2c is half of that found in exp 2b or 2d.

The effect of spawn type on mycelial colonization and mushroom yield has been documented. Rosdao et al (2002) found that liquid spawn resulted in a “more homogeneous fungal growth”. If grain spawn is used the grain type as well as the spawn rate (weight of spawn per weight of substrate) can influence yield (Bhatti et al 2007, Nwanze et al 2005, Sangeetha et al 2008). While the studies compared effect of spawn grain type on yield there was no mention FIB. Future research is needed to substantiate our finding in the differences in yield and FIB based on the use of two different types of grain spawn.

3.5.4 Inverse relationship between yield and FIB

We suspected that higher fruiting in the bag would be correlated with lower mushroom yield. Experiment 2d (four *Hericium* isolates, one sawdust type, rye grain spawn) in particular demonstrated an inverse relationship

between harvestable yield and FIB weight. Experiment 2b (four *Hericium* isolates, two sawdust types, rye grain spawn) showed this inverse relationship for two out of the three wild isolates being tested. This suggests that high FIB is detrimental to the yield. We can hypothesize that energy is wasted creating malformed mushrooms inside the growing bag (FIB) and that if FIB could be reduced, that energy would be allocated towards producing harvestable fruiting structures on the outside of the bag (yield).

The inverse relationship between yield and FIB was not shown for any isolate in exp 2c (four *Hericium* isolates, to sawdust types, millet grain spawn). One reason no significant relationship could be detected for weight of yield and FIB in exp 2c could be that the data did not display a good range of values. In general, the bags produced far lower yields of mushrooms compared to the other experiments. Most of the data points fall in the high FIB low yield range. No yield from a single bag was over 120g in exp 2c while exp 2b and 2d included bags with yields above 300g and 400g. Since no bags produced a high yield of mushrooms we cannot determine what the corresponding FIB weights would have been. Without a wide range in values it is more difficult to determine the relationship between the two factors. Since only exp 2c used millet grain spawn, this suggests that the spawn type may have some effect on the yield and FIB weights.

The relationship between yield and FIB was not ever shown to be significant for the commercial isolate, FFP3. The lack of a relationship for the commercial isolate, FFP3, has a similar explanation as that for exp 2c. For experiment 2b and 2d no FFP3 bags produced over 50g and 75g of fresh weight FIB, respectively. Again since the data for this isolate did not include a range in values of FIB similar to that of the other isolates, which had bags with

FIB values up to 330g and 480g, we cannot discount the possibility that there would be a relationship if we had observed a FFP3 bag with high FIB (300-500g). On the other hand, since no FPP3 bags with high FIB were observed it could also be suggested that FIB is not a substantial problem for the commercial isolate. In exp 2c, where millet grain spawn was used, FFP3 did not perform significantly differently in yield or FIB from the other isolates and no relationship between yield and FIB could be detected. This again suggests that the type of grain spawn used is affecting the yield and FIB.

3.5.5 Total weight yield

In general, when harvestable yields and FIB weights are combined (total yield) there were no differences between the commercial and wild isolates. There are problems with combining yield and FIB weights to create a "total weight yield" variable and the suggestion that solving the FIB problem would cause that same mass to be converted to harvestable mushrooms is arguable. The structures that form inside the bag do not appear to be properly formed mushrooms and therefore their size/weight may be exaggerated to the actual size/weight of the mushroom if it had been able to properly form. Despite this issue it is still important to note that, in general, the total dry and fresh weight yield of the wild isolates is not different from that of the commercial isolate. This suggests that if the FIB issues can be resolved the wild isolate will have more resources to give to the formation of properly formed harvestable mushrooms (yield). If this is indeed true, it would be interesting to further investigate methods to reduce the occurrence of FIB as a way to increase the harvestable yield of mushrooms from these wild isolates.

FIB could be reduced in several was. First, the setup used for these

experiments utilized bags that had a large amount of air space. This is the area where the malformed mushrooms generally formed. We used these bags because they were similar to the bags used by a commercial *Hericium* grower (Phillips Mushroom Farm). Another option would have been to use a cylindrically shaped polypropylene bag. These bags are filled with the sawdust substrate and spawn is added to the top without being mixed in or the spawn is mixed into the sawdust before it is put in the bag. The top of the bag is then cinched together so that no air space is at the top of the bag. The hole is plugged with a sterile cotton plug. A similar set up uses plastic bottles filled with substrate. After sufficient mycelial colonization the plug is removed and mushrooms are allowed to form (Stamets 2000). This model does not allow room for FIB to form. If resources could be diverted from forming FIB in this way it is reasonable to hypothesize the wild isolates would produce yields closer to that of the commercial isolate. In a study by Chang & Roh (1999) higher yield was found when *H. erinaceus* was grown in bottles as compared to bags. High FIB in the bags was not mentioned but this could have possibly been a contributing factor.

Another possibility for reducing FIB is to alter the concentration of CO_2 in the production rooms. High concentrations of CO_2, typically 1,000 - 5,000 ppm (as high as 40,000 ppm in some sources), are suggested to suppress mushroom formation until the holes are poked in the bags; at which time, the CO_2 levels are drastically reduced (Ko 2005, Xiao 1997, Figlas 2007, Stamets 2000) While data on CO_2 concentrations were not regularly collected for our experiments, concentrations ranged between 500-700 ppm. Perhaps by simply increasing the CO_2 concentrations in the growing room while the mycelium is colonizing the sawdust in the bags, FIB produced by the wild

isolates may be reduced. While the bags do have a small breathable patch that is designed to allow gas exchange, it is questionable as to how much gas exchange is actually allowed by such a small patch. It would be interesting to examine the CO_2 concentrations inside the bags compared to the CO_2 concentrations in the growing room. Donoghue and Dension (1995) explored patch size related to yield and CO_2/O_2 concentrations in the bags for shiitake (*Lentinula edodes*) production. The researchers found CO_2 and O_2 concentrations inside the bag was dependent on filter size, with larger filter size resulting in higher O_2 levels and lower CO_2 levels. Interestingly, the response of yield to the different gas compositions was dependent on fungal strain.

Since there was no addition of CO_2 to the growing room for this experiment, differences in production of FIB among the isolates may be related to differences in the amount of CO_2 being produced by each isolate. A study by Grigansky et al. (1999) determined that certain *Hericium* isolates produced significantly different maximum CO_2 concentrations. This may explain why the wild isolates (He 1, He 2 and He 4) had greater FIB compared to FFP3. If high CO_2 concentrations suppress primordia formation it is reasonable to expect that the wild isolates are not producing as much CO_2 compared to the commercial isolate (FFP3) therefore, the concentration of CO_2 is lower in these bags and primordia formation is not suppressed.

The study also suggests that measuring the amount of CO_2 being produced by a specific bag could aid in determining the proper time to move the bag to a second room (fruiting room) with lower temperature and CO_2 concentrations to induce fruiting. The researchers suggested that when CO_2 production hits a maximum and begins to drop, the substrate has been fully

colonized and fruiting should be initiated. This technique would be useful in future experiments with these wild isolates to give a objective protocol for determining when a bag of a specific isolate is ready to be moved from the growing room to a second fruiting room.

3.5.6 Postharvest: Fresh weight loss

A fresh weight loss of as little as 3-6% can cause marked deterioration of mushroom quality (Sveine et al 1967). While differences in weight loss can be detected between the mean percent weight loss of mushrooms by isolate when the data was analyzed by substrate, all the mushrooms had a percent weight loss of more than 3-6% in the first three days of storage. This acceptable range in weight loss was based on the common button mushroom (*Agaricus bisporus*), the focus of most of the current research. In a study of oyster mushrooms 2% postharvest weight loss was seen after 11days, which was more than the researchers were expecting to find (Villaescusa 2003). The *Hericium* mushrooms in our study lost between 12 and 15% within the first 3 days. As specialty and gourmet mushrooms become more popular among consumers research focused on these specialty mushrooms is required to maximize yield and storability of these specialty mushrooms. Since all the *Hericium* mushrooms (including the commercial isolate, FFP3) lost over 3-6% weight perhaps the percent weight loss that can be tolerated for *Hericium* mushrooms is higher or the storage protocol used for other mushrooms is not adequate for *Hericium* mushrooms.

While differences in percent weight loss between substrates were not found to be statically significant, the interaction of substrate x isolate was. The commercial isolate had higher weight loss when grown on Fg sawdust

and conversely He 1 had higher weight loss when grown on Ar sawdust. The other two isolates appear unaffected by substrate. It is difficult to explain this.

Weight loss is from respiration and water loss. If an isolate produces smaller mushrooms when grown on one substrate these smaller mushrooms have higher surface area: volume ratio and therefore lose water at a faster rate. This was not found to be the case. Alternately, perhaps a certain isolate's ability to digest/extract nutrients from wood is dependent on isolate and wood type. The chemical makeup of the mushrooms produced on different wood types could affect the chemical makeup of mushrooms, thus causing some to decompose faster thus losing more weight through respiration.

3.5.7 Postharvest: Quality Rating

The block (shelf in which the mushroom was grown) in each experiment was significant. This shows that the block where the mushroom was grown had an effect on the quality of the mushroom. The lower shelves probably had more air flow which would dry out the mushrooms produced on those shelves. The fogging machine produced fog above the top shelves, so the mushrooms there most likely had a higher moisture content which would cause them to bruise more easily. The effect of block was accounted for in the models.

The sawdust substrate was not a significant factor for predicting mushroom quality; however the interaction between isolate and substrate was significant in most cases for most days. We separated the data by substrate before comparing the overall quality ratings of the mushrooms by isolate.

It is important to examine the quality of the mushrooms for a wide range of days. Fresh mushrooms have a short shelf life and typically reach the

consumer 2-3 days after they are picked. For days 0 and 3 we found that, regardless of experiment or substrate, in most cases there were differences in quality among the isolates. Where differences were seen it was typically wild isolates He 1 and He 4 being of lower quality to the commercial isolate, FFP3. This was similar to quality ratings on day 6. By day 9 there were generally no differences in quality rating among the isolates. In cases where differences were seen, again the commercial isolate had better quality rating compared to some or all of the wild isolates.

There were rarely (in only one case) differences among the wild isolates. In some cases, mushrooms produced by FFP3 did have higher quality compared to the wild isolates and sometimes there was no difference in the quality ratings. However, it is true that the wild isolates never produced mushrooms that had better quality than those of FFP3.

Observations of the researcher during the collection of the quality data gave the impression that distinct differences among the isolates would be found for all days. Either the impression was wrong or the subjective quality rating scale was not discriminating enough to allow the differences to be detected. Perhaps a subjective scale that separated the rating by 8 or 10 levels would give a better representation of the quality of each mushroom.

The subjective rating scale created for these experiments primarily evaluated percent of the mushrooms that was dried or discolored. This scale did not take into consideration other general appearance characteristics like size or shape of the mushrooms. Perhaps there was truly no difference between the amount of surface drying and the impressions of the researchers were due to the general misshapenness or size of mushrooms produced by certain isolates. Research exploring the visual characteristics customers value

most may aid in creating a more useful quality rating scale. The results that no consistent difference in quality rating exists between the isolates cannot simply be discounted; future experiments using improved quality rating scales may be useful when determining quality characteristics of the mushrooms.

Subjective rating scales used in conjunction with trained consumer panelists have been successful in other studies investigating quality of mushrooms (VIllaescusa 2003). A study by Ares et al. (2006) used a consumer panel and a “sensory intensity scale” to rate characteristics like odor, firmness, and cap color of oyster mushrooms under different storage treatments. In addition, they objectively determined shelf-life of mushrooms by determining the number of days of storage that resulted in a 25% rejection of mushrooms by a consumer panel.

3.5.8 Sister experiment at Phillip’s Mushroom Farm

A sister experiment, similar to exp 2b, 2c, and 2d was conducted by Phillips Mushroom Farm, a commercial mushroom production company. Data was collected on yield, percent weight loss and quality. This experiment utilized the six wild collected isolates (He 1 - He 6), the commercial isolate (FFP3) and the commercial isolate of *H. erinaceus* used by Phillips Mushroom Farm (P104). The experiment was run twice. Specifics on this experiment can be found in the appendix of this document.

The data couldn’t be analyzed statistically because yield was not recorded for each bag and the bag to bag variability is unknown. Average yield per bag was calculated by dividing the weight of yield from all the bags of an isolate by the number of bags (24). The fresh weight yield data for the isolates which were included in exp 2b, 2c and 2d (He 1, He 2 and He 4) appear to be

similar to the our results from those experiments. In each case the average fresh weight yield of the wild collected isolates (He 1, He 2 & He 4) was less than the yield of the commercial isolate (FFP3). However, the other wild collected isolates (He 3, He 5 and He 6) sometimes had fresh weight yields that were more comparable to both commercial isolates (FFP3 and P104). While there was not a wild isolate that far surpassed the yield of the commercial isolate, it is encouraging that out of such a small sample size of wild isolates (6), some appeared to produce relatively high yields.

Overall, the weight loss data from the Phillips experiment appears to be similar to our weight loss data of exp 2b, 2c and 2d. The commercial *H. erinaceus* strains (FFP3 and P104) had lower weight loss compared to the other wild *H. americanum* strains.

As with exp 2b, 2c and 2d the average weight loss of mushrooms in the Phillips experiment on day 3 was between 7% and 14% which was well above the 3-6% that is reportedly not tolerated for *Agaricus* mushrooms (Sveine et al 1967). For the Phillips experiment weight was taken every day for 8 days and the reported weight loss for day one was actually between 3-6%. Even the mushrooms from the isolate currently being used for production had a weight loss of at least 3% on day 1 and 6% by day 2. Since these are the mushrooms currently being sold commercially it seems that these weight loss figures should be considered the standard acceptable weight loss for *Hericium* mushrooms. *Hericium* mushrooms have a very high moisture content (80-90%) so perhaps a slight shrinkage of the mushroom improves the textural quality slightly, preventing consumers from getting “water logged” mushrooms. Alternatively, perhaps the availability of high quality *Hericium* mushrooms is so sparse that the consumers simply do not know any better and accept a lower

quality product. Further research is required to determine what percent weight loss is acceptable for *Hericium* mushrooms. A useful study would be to determine at what percent weight loss detrimental texture differences can be detected by subjects in an organoleptic test.

The quality information collected for the Phillips experiment consisted simply of comments by the head grower on the general appearance and quality of the mushrooms. The commercial strain, FFP3, was reported to have quality equal to that of the commercial strain currently being used by Philips (P104). However, for the most part the comments were not favorable for the wild isolates. Most of the comments suggested the mushrooms from the wild isolates were “soft”, “coral or cauliflower like”. Out of the six wild isolates only mushrooms from He 3 and He 4 “could be salable”. The wild isolates were all *H. americanum* which produce mushrooms that have a more coral-like and branching appearance compared to the mushrooms of *H. erinaceus,* which are much more compact. Photographs of mushrooms sent from the Phillips experiment shows the appearance of the *H. americanum* mushrooms do not differ from those grown for experiments 2b, 2c and 2d. It seems the growers simply prefer the more compact appearance of the *H. erinaceus* isolates. It would be useful to see if consumers would be accepting of the branching coral-like form of the *H. americanum* mushrooms before further research on production is conducted.

3.5.9 Totem experiment

The low yields and low percentage of mushroom producing logs after one year of collection cannot be taken as a sign of a failed experiment; these data should be looked at as preliminary results. Mushroom logs can produce

mushrooms for 5+ years and observations from other (unpublished) experiments conducted at Cornell University show that logs inoculated with *Hericium* isolates do not produce sizable yields until 3 years after inoculation with spawn. These observations that logs inoculated with *Hericium* spawn do not produce mushrooms until the log is fairly decomposed suggests that *Hericium* prefers highly decomposed wood and may take advantage of wood that has been partially broken down by other fungus. To further support this hypothesis, it is in our experience that wild *Hericium* mushrooms are most commonly found on well rotted logs. When collecting mushrooms to create cultures for this experiment many of the logs producing the mushrooms were so decomposed the tree species could not be determined.

Actual yield could not be compared statistically; however, it is encouraging that the highest percentage of logs producing mushrooms was one of the wild collected isolates. While we cannot be positive this trend will continue in the future the current data seems to support the hypothesis that this wild isolate will outperform the commercial isolate. This may be attributed to the fact that the locally collected isolates are better adapted to the climate in which they were collected compared to a presumably foreign collected isolate (FFP3).

3.6 Conclusions

Since the hypothesis that fast *in vitro* growth rate would be found in the isolates with the highest yield was rejected, *in vitro* growth rate on PDA cannot be recommended to screen newly collected wild isolates. In order to conduct an evaluation of a large number of wild isolates in a timely manner, it is imperative to find an *in vitro* characteristic that can be used as a screening

tool. Further experiments involving linear growth tests may prove a relationship between fast mycelial growth on sawdust substrate and high yields.

Even though exp 2b, 2c and 2d showed that the wild isolates had lower yield compared to the commercial isolate (FFP3), it must be considered that this is only three randomly collected isolates out of the countless isolates that exist in the wild. When all six wild collected isolates compared with the commercial isolates in the Phillips experiment, some of the wild isolates seem to produce yields that are comparable to the commercial isolates (FFP3 and P104). The fact that none of the wild collected isolates produced yields greater than that of the commercial isolates should not be taken as a reason to end further pursuit of wild isolates in commercial production. Six isolates out of the countless number of isolates in the wild is a very small sample size. The search for high yielding wild isolates cannot be abandoned until a very large number of isolates are collected over a wide area.

The wild isolates collected for this study had higher FIB compared to the commercial isolate and often demonstrated an inverse relationship between FIB and yield. High FIB can be considered a production problem, at least for the wild isolates. Further research should be focused on testing methods designed to reduce the occurrence of FIB.

When weight of dry yield and FIB are combined (total yield) there is no difference between the commercial isolates and the wild isolates. If FIB could be reduced, it is hypothesized that more energy would be allocated to the formation of properly formed harvestable mushrooms and the yields of the wild isolates could equal those of the commercial isolates. This further demonstrates the importance of researching methods designed to reduce the

occurrence of FIB.

Since all the isolates tested produced mushrooms that lost a high percentage of weight postharvest, additional research on postharvest characteristics of *Hericium* mushrooms is needed. The relationship between mushroom weight loss and textural quality of the mushroom would aid in determining what range of weight loss can be tolerated for *Hericium* mushrooms. Creation of a quality rating scale that incorporated not only evidence of aging (drying and discoloration) but also general characteristics of appearance (size and shape) would aid in objectively rating mushrooms produced by different isolates.

The best prospect for wild *Hericium* isolates in commercial production appears to be in outdoor production. While yields could not be compared this early in the experiment the highest percentage of logs producing mushrooms was one of the wild collected isolates. This trend appears to be continuing with the wild isolates producing large mushrooms in the fall of 2009 (data in appendix 4). We suspect this is because the wild isolates are better adapted to the local environment which may give them an advantage over the commercial isolate of *H. erinaceus* (FFP3). Also, the commercial strain has been selected for commercial use because it performs well when grown indoors on sawdust. This does not necessarily mean that it will also perform well when grown outdoors on logs.

Specialty mushrooms are surely a growing industry and in the United States, little research about mushroom production, storage and quality is currently being conducted on specialty mushrooms species. Collection and evaluation of wild isolates may be a source of new isolates which produce high yields of high quality edible mushrooms.

APPENDIX 1

Making sawdust mixture when 500g dry sawdust is required

Proportions of ingredients

0.060 H2O

0.01 $CaSO_4$

0.078 wheat bran

0.312 sawdust

1. Determine total weight of mixture with 500g DRY sawdust

500g = (t) 0.312

(t)=total weight of mixture

2. Determine weight of $CaSO_4$ and wheat bran

(c)= (t) 0.01

(c) = weight of $CaSO_4$ to add

(b) = (t) 0.078

(b)= weight of wheat bran to add

3. Determine amount of fresh sawdust to add

Place 100 g of fresh sawdust in drying oven for 24 hours

DW sawdust = weight of 100g fresh sawdust after drying

100g fresh sawdust- DW sawdust = % moisture of sawdust

500g / DW sawdust = (s)/100g fresh sawdust

(s)= amount of fresh sawdust to add to mixture

4. Determine the amount of H_2O

(w_1)= (t) (0.60)

(w_1) = amount of water required for mixture

** remember there is already H_2O in the fresh sawdust**

(s) – 500g = (w_2)

(w_2)= amount of H_2O in fresh sawdust

(w_1)- (w_2) = (w_0)

(w_0)= amount of water to add to mixture

APPENDIX 2

Raw data from exp 2.0 trial experiment

The wild isolate He 5 produced no mushrooms on unsupplemented sawdust.

Table A2.1 Mean fresh weight of *Hericium* mushrooms produced on two different unsupplemented sawdust substrates, Fg (*Fagus grandifolia*) or Ar (*Acer rubrum*) sawdust.

Isolate	Substrate	Weight (g)
He 5	Ar	0
	Fg	0
FFP3	Ar	23.00
	Fg	18.33

APPENDIX 3

Comparison of seven *Hericium* isolates at Phillips Mushroom Farms Kennett Square PA, USA

Methods and Materials

Frozen cultures of all seven isolates (He 1 – He 6, Hericium americanum, and FFP3, Hericium erinaceus) were sent to researchers at Phillips Mushroom Farms. After receiving the culture vials, the samples were plated onto PDA (Oxoid) and allowed to grow out for 8 days. These cultures were then used to inoculate gallon sized "master" bottles filled with sawdust substrate. The masters were given two shakes about 4 days apart. Before fully colonized, the bottles were sent to the cooler for storage until needed. A master created from the *Hericium erinaceus* isolate that is used by Phillips Mushroom Farm (PMF104) was created according to the same procedure and used in this experiment.

These masters were then used to grow mushrooms in the same manner Phillips Mushroom Farm commercially produces *Hericium* mushrooms. One master of each isolate was used to inoculate sawdust substrate in 24 bags weighting approximately 2.5 kg. The sawdust substrate was sterilized and had post-sterilization moisture of 58.07% and a pH 5.92. Bags were allowed to colonize the sawdust substrate (spawn run) at 18-19°C, 80% humidity and 2,000 – 2,500ppm CO_2 for 9 days before being sent to the growing room. At this point eight holes were placed in each bag to allow mushrooms to grow out from the plastic bag. The growing room conditions were set at 14-16^{0}C, 95% relative humidity and 600-800ppm CO_2. Mushrooms were picked as they appeared daily during the first flush. After this first flush of mushrooms the

sawdust bags were discarded. An abbreviated mushroom quality study was completed by recording data for only a small sample of mushrooms. Three trays were set up with one or two mushrooms per strain on each tray. The mushrooms were stored in the facility cooler and were weighed each day for eight days. Quality comments referencing the visual quality of the mushrooms were recorded. The entire experiment was run twice.

Results

Experiment 1

Table A3.1 Total yield of mushrooms and average yield of mushrooms per bag for two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum*(He1, He2, He3, He4, He5, He6): Phillips exp 1

Isolate	total yield (g)	average yield (g)
P104	9094.5	378.9
He 1	6617.9	275.7
He 2	3451.8	143.8
He 3	6813	283.9
He 4	2635.4	109.8
HE 5	7112.3	296.3
He 6	7429.8	309.6
FFP 3	8922.2	371.8

Table A3.2 Average percent weight loss (g) (3 per treatment) of mushrooms from two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum* (He1, He2, He3, He4, He5, He6): Phillips exp 1

Isolate	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8
P104	3.4	6.6	9.6		16.6	19.7	23.0	26.3
He 1	3.5	8.5	12.3		19.8	23.6	27.8	31.1
He 2	4.1	7.7	12.3	15.1		22.9	25.9	28.9
He 3	2.7	8.6	13.6	16.9		24.5	28.7	32
He 4	4.3	8.2	14.2	18		16.4	30.9	34.9
He 5	3.6	8	12.3	16.1	20		27.7	31.5
He 6	4.1	7.8	13.2	16.6		25.2	28.5	31.5
FFP3	3	5.6	7.8		12.9	15.5	18.8	21.6

Table A3.3 Quality comments for mushrooms produced by two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum* (He1, He2, He3, He4, He5, He6): Phillips exp 1

Isolate	COMMENTS
#104	Current production strain looks normal.
#121	Good size but ugly, cauliflower like heads and yellowish color.
#122	Size is fine but still has that cauliflower look though not as bad as #121
#123	Size is fine quality is OK, could be salable.
#124	Size is good, a little bit more cauliflower like but as it got older it looked better. Not bad.
#125	Big heads but not good looking
#126	Very close to 124. Size is good but still has that cauliflower look to it.
#130	This is as close to our normal strain as there is in this group. Looks fine. Salable, a good second strain.

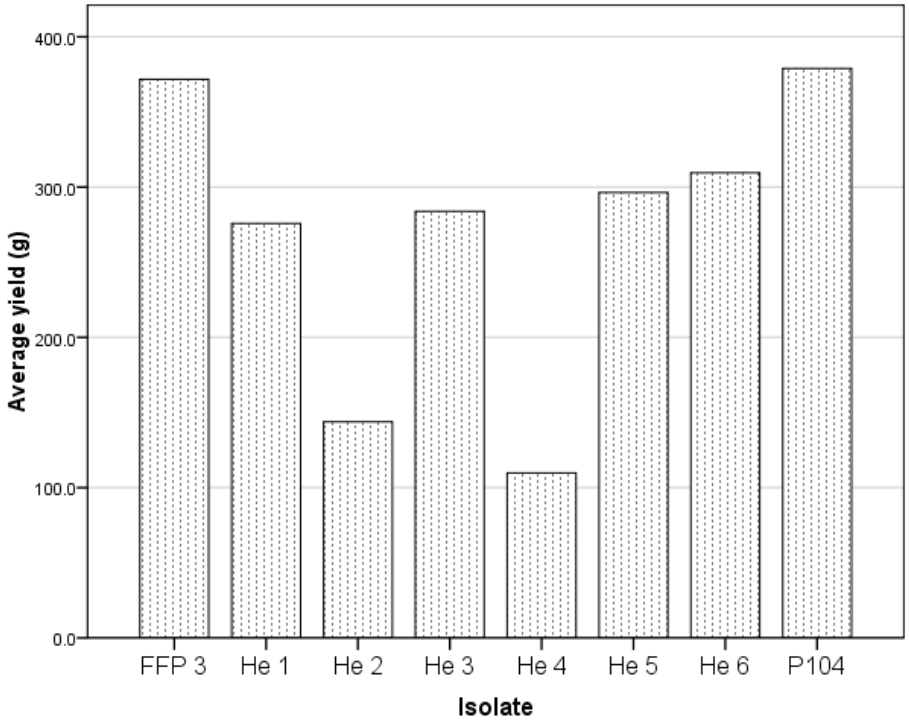

Figure A3.1 Comparison of average yields of mushrooms per bag for two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum* (He1, He2, He3, He4, He5, He6):Phillips exp 1

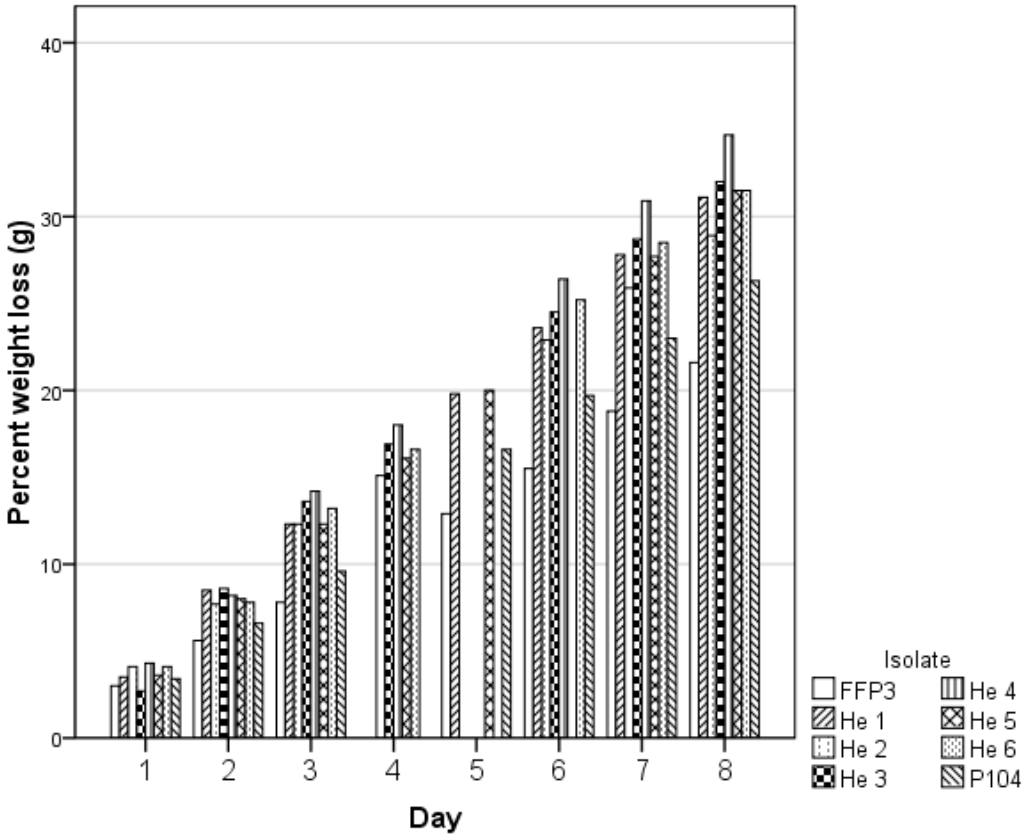

Figure A3.2 Comparison of average weight loss of mushrooms from two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum* (He1, He2, He3, He4, He5, He6) over 8 days: Phillips exp 1

Experiment 2

Table A3.4 Total yield of mushrooms and average yield of mushrooms per bag for two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum* (He1, He2, He3, He4, He5, He6): Phillips exp 2

Isolate	total yield (g)	average yield (g)
P104	5393.2	224.7
He 1	5334.2	222.3
He 2	4907.9	204.5
He 3	5198.2	216.6
He 4	3574.3	148.9
He 5	5992	249.7
He 6	3161.3	131.7
FFP 3	9675.1	403.1

Table A3.5 Average percent weight loss (g) (3 per treatment) of mushrooms from two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum* (He1, He2, He3, He4, He5, He6): Phillips exp 2

Isolate	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8
P104	3.1	6.8	10.8	15.4		26.1	31.8	36.8
He 1	6.7	14.7	24.5	31.4		43.1	48.3	53.2
He 2	2.8		9.1	13.6	16.8	21.8	26.2	31.2
He 3	6.8	13.9	21.8	28.9		40.9	46	50.9
He 4	6.3	12.4	18.4	24.3		34.3	38.7	42.8
He 5	3.2		10.8	14.7	18.6	23.2	28.6	32.7
He 6	5.3	9.8	15.6	19.8		27	30.3	33.3
FFP3	2.8		10.1	12.7	16.2	19.4	24	27.8

Table A3.6 Quality comments for mushrooms produced by two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum* (He1, He2, He3, He4, He5, He6): Phillips exp 2

ISOLATE	COMMENTS
P104	Normal looks fine.
HE 1	A little off color (darker) very irregular, loose and soft, not too good.
He 2	Better color, but same quality as #121, not good.
He 3	Soft coral-like shape. Good size but still not great quality.
He 4	Same as #123.
He 5	Coral like, very loose/open/soft, not too good.
He 6	Same as #125, very similar, not good.
FFP 3	Firm, a little irregular in shape, but very close to #104 should be saleable.

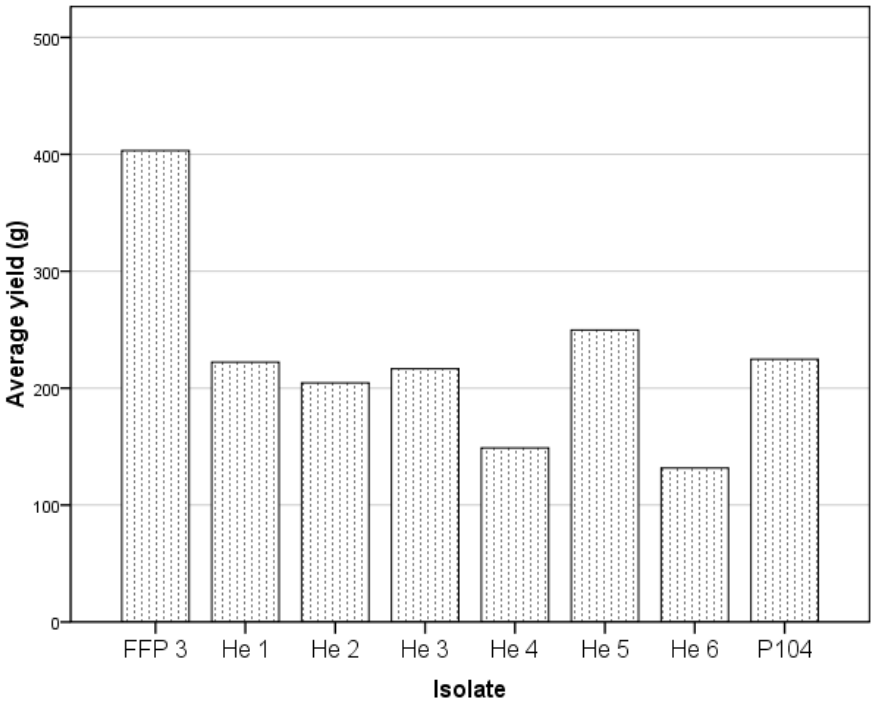

Figure A3.3 Comparison of average yields of mushrooms per bag for two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum* (He1, He2, He3, He4, He5, He6):Phillips exp 2

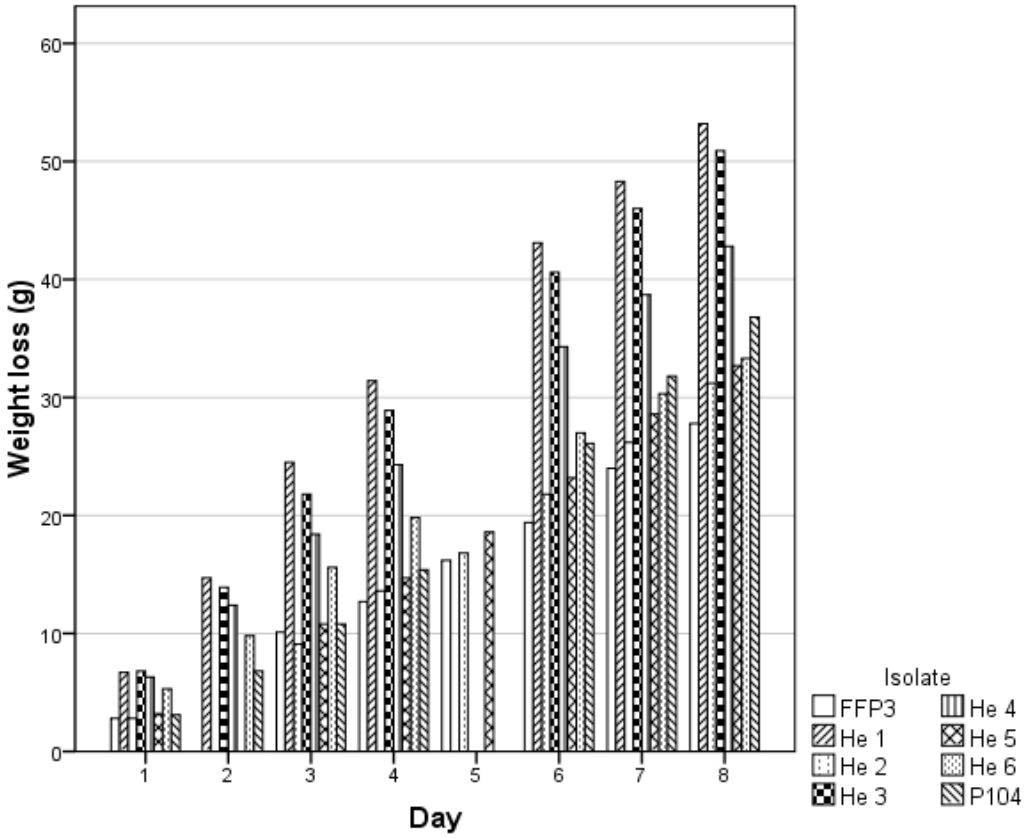

Figure A3.4 Comparison of average weight loss of mushrooms from two commercial isolates of *Hericium erinaceus* (P104 and FFP3) and 6 wild collected isolates of *Hericium americanum* (He1, He2, He3, He4, He5, He6) over 8 days: Phillips exp 2

APPENDIX 4

These graphs represent data collected from experiment 3 between April 2008 and October 2009. Figure A4.1 shows the percent of logs that produced at least one *Hericium* mushroom (successfully fruited). While these values were not compared statistically, it appears that the wild isolate He3 has a greater percentage of logs that successfully fruited. Figure A4.2 shows the average yield (g) of mushroom produced per isolate. While these values were not compared statistically, it appears that the wild isolates He3 and He5 have remarkably higher yields compared to the commercial isolate, FFP3, and one of the other wild isolates, He4.

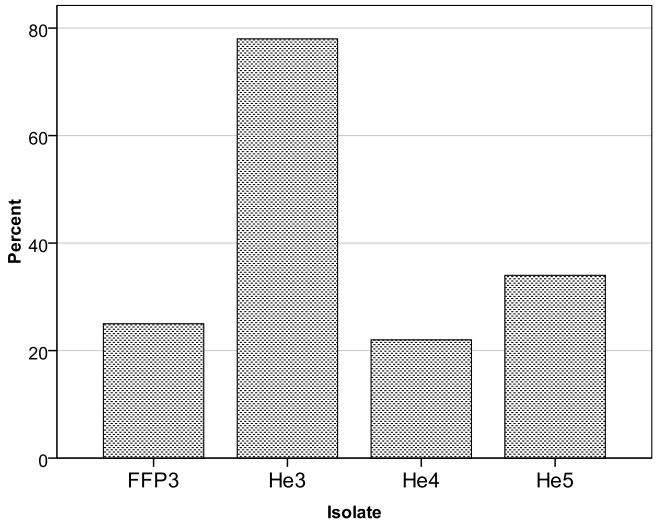

Figure A4.1 Comparison of percent logs which produced at least one *Hericium* mushroom between April 2008 and October 2009 for four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) for exp 3

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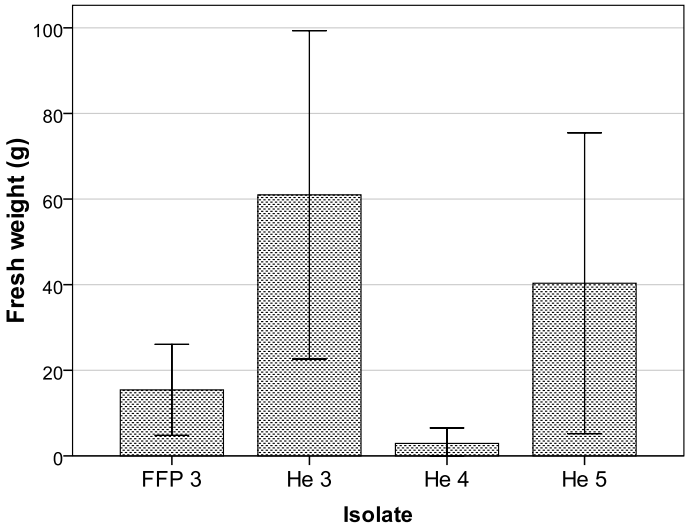

Figure A4.2 Comparison of mean fresh weight of *Hericium* mushroom produced by four *Hericium* isolates (FFP3, a commercially grown *H. erinaceus* isolate and He1, He2 & He4 wild collected *H. americanum* isolates) on logs for exp 3

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